GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

Founded in 1916
by
GEORGE H. SHULL
with the cooperation of

WILLIAM E. CASTLE
EDWIN G. CONKLIN
CHARLES B. DAVENPORT
BRADLEY M. DAVIS
EDWARD M. EAST
ROLLINS A. EMERSON
HERBERT S. JENNINGS
THOMAS H. MORGAN
RAYMOND PEARL

Volume 39 January, 1954 Number 1

TABLE OF CONTENTS

PLOUGH, HAROLD H., Edwin Grant Conklin 1

STALKER, HARRISON D., Parthenogenesis in Drosophila 4

CASTLE, W. E., Coat color inheritance in horses and in other mammals 35

SLATIS, HERMAN M., AND DAVID A. WILLERMET, The miniature complex in *Drosophila melanogaster* 45

SWAMINATHAN, M. S., Nature of polyploidy in some 48-chromosome species of the genus Solanum, section Tuberarium 59

BRNCIC, DANKO, Heterosis and the integration of the genotype in geographic populations of *Drosophila pseudoobscura* 77

CAIN, A. J., AND P. M. SHEPPARD, Natural selection in Cepaea 89

AVERS, CHARLOTTE J., Chromosome behavior in fertile triploid Aster hybrids 117

NOVITSKI, EDWARD, The compound X chromosomes in Drosophila ... 127

PUBLISHED BIMONTHLY AT LANCASTER, PENNSYLVANIA,
BY GENETICS, INC.,
GENETICS BLDG., UNIVERSITY OF WISCONSIN, MADISON 6, WISCONSIN

(Date of issue, March 2, 1954)

EDITORIAL BOARD

R. ALEXANDER BRINK, *Managing Editor,*
JAMES F. CROW, *Associate Managing Editor,*
University of Wisconsin

GEORGE W. BEADLE
California Institute of Technology

WILLIAM E. CASTLE
University of California

BRADLEY M. DAVIS
University of Michigan

L. C. DUNN
Columbia University

JOHN W. GOWEN
Iowa State College

DONALD F. JONES
Connecticut Agricultural Experiment Station

M. M. RHOADES
University of Illinois

GEORGE H. SHULL
Princeton University

T. M. SONNEBORN
Indiana University (Representative of the Genetics Society of America)

L. J. STADLER
U. S. Dept. Agriculture, University of Missouri

CURT STERN
University of California

ALFRED H. STURTEVANT
California Institute of Technology

SEWALL WRIGHT
University of Chicago

Volume 39 JANUARY, 1954 Number 1

GENETICS is a bimonthly journal issued in annual volumes of about 600 pages each.

Subscription, $8.00 net a year for complete volumes (January-November). Foreign postage, 50 cents additional. Single copies, $1.50 each, postpaid.

As available, back volumes may be had at $14.00 each and single issues of previous volumes at $2.50 per copy, postpaid. The Science Press will supply information on request as to volumes and numbers available.

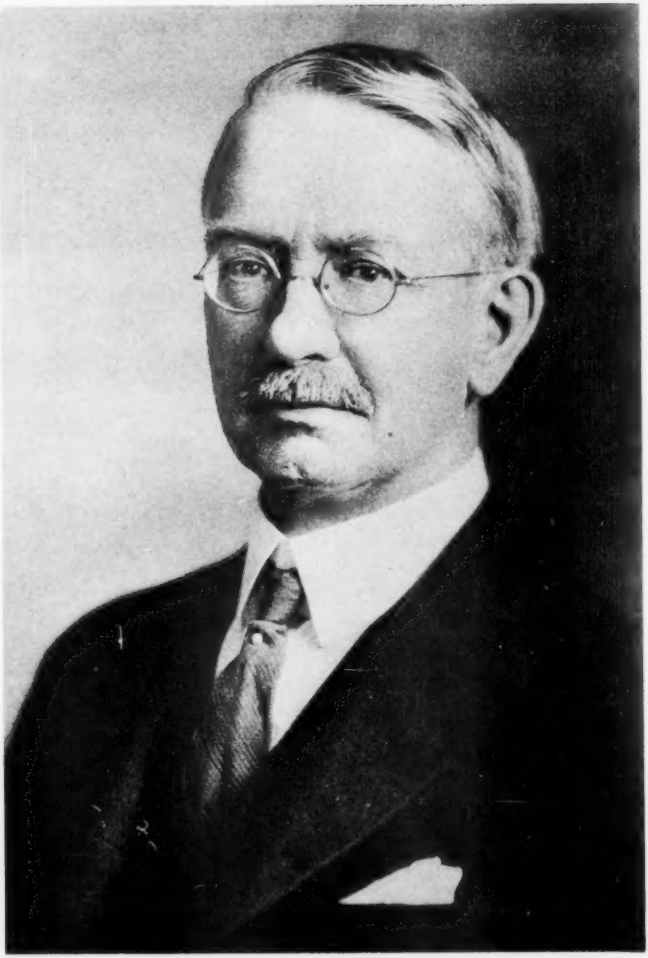
Business Correspondence, including change of address, etc., should be addressed to GENETICS, INC., THE SCIENCE PRESS, Box 749, Lancaster, Pa.

Remittances should be made payable to GENETICS, INC.

Correspondence concerning editorial matters should be addressed to the EDITORS OF GENETICS, Genetics Building, University of Wisconsin, Madison 6, Wisconsin.

Entered as second-class matter at the postoffice at Lancaster, Pa., June 14, 1951, under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in the Act of February 28, 1925, paragraph 4, section 412, P.L.&R., authorized January 9, 1932.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.



Sincerely yours,
E. J. Conklin

EDWIN GRANT CONKLIN[1]

1863–1952

THE death of PROFESSOR EDWIN G. CONKLIN of Princeton University on November 21, 1952 brought to a close the long career of a first-rate investigator and a great teacher in biological science. He was known and admired by generations of Princeton students, but by reason of his wide knowledge and his remarkable memory, his effectiveness on the lecture platform, his numerous friendly scientific contacts at Woods Hole, in the American Philosophical Society, the National Academy of Sciences, and in all of the major American biological societies, he became and remained for many years the most effective American interpreter of genetics and evolutionary problems from the investigator to the intelligent public. His own special interest was the field of embryological development and especially differentiation, but the pursuit of the mechanism by which an undifferentiated egg becomes an embryo and an adult displaying characters of both parents led him inevitably to genetics. He was a proponent and a friendly critic of the basic discoveries of chromosome behavior of E. B. WILSON and of the fundamental genetic experiments of the Drosophila group under T. H. MORGAN. By his public lectures and his books, especially "Heredity and Environment" (1914), which went through six editions and was translated into Russian and Japanese, he attained popular recognition as an important authority in the field of genetics, even though he never carried on genetic studies nor published a paper in the field. So general was the recognition of his competence that he was chosen for the editorial board of GENETICS when it was established in 1916 and served until his death. DR. G. H. SHULL, who established the journal and was its managing editor for so many years, says that DR. CONKLIN was a particularly faithful member and, indeed, that it was he who suggested the name GENETICS. His only direct contribution to the journal was an article on LAMARCK which accompanied the portrait frontispiece in Vol. 29 (1944).

In an article entitled "Mosaic vs. Equipotential Development" published in *The American Naturalist* in 1933, the year of his retirement from the chairmanship of the Department of Biology at Princeton, PROFESSOR CONKLIN wrote: "Throughout my scientific life I have been waging a fight for the recognition of the importance of the cytoplasm of the egg," and this indeed was the central theme of his career as an investigator. It began with his doctoral thesis in 1891 under PROFESSOR W. R. BROOKS at Johns Hopkins (published in 1897) on the cell lineage of Crepidula, and continued with his important studies of the "Organization and cell lineage of the Ascidian egg" in Styela

[1] Portrait by ORREN JACK TURNER, Princeton, New Jersey.

(1905) and his successive reports of his experiments on the developing eggs of Amphioxus. He emphasized over and over again (as on page 197 in "Heredity and Development," 3rd ed. 1920) that "at the time of fertilization the hereditary potencies of the two germ cells are not equal, the polarity, symmetry, type of cleavage, and the pattern or relative positions and proportions of future organs being foreshadowed in the cytoplasm of the egg cell, while only the differentiations of later development are influenced by the sperm." This appeared to lead him to the false position already defended by LOEB (1916) that the "embryo in the rough" is determined by the cytoplasm alone, and only the finer details are added by the influence of the genes in the nucleus. As WILSON (1920) soon pointed out, however, the pattern and other details of early development are influenced by the genes of the female parent, derived from its parents, so the action of the genetic determiners is simply pushed one generation back. This corrected interpretation was accepted by CONKLIN and incorporated in later discussions. His continued emphasis on the problems and mechanisms of differentiation, including the importance of environment, have borne fruit in the more recent discoveries classified under the heading of physiological genetics.

Another area in which DR. CONKLIN showed a continuing and fertilizing interest was the mechanism of evolutionary change, since DARWIN and WEISMANN, one of the major theoretical problems which all biologists feel compelled to discuss. In May 1896 the American Philosophical Society held a symposium on the subject "Factors of Organic Evolution" at which the speakers were PROFESSORS E. D. COPE, LIBERTY H. BAILEY, and the youthful CONKLIN. COPE defended the Lamarckian view; BAILEY believed that variability is the inherent condition of organisms, and environment eliminates the nonvariable, while CONKLIN followed the Weismannian argument that acquired characters are not inherited, but hereditary variations are determined by changes in submicroscopic units in the germ cells induced by extrinsic forces, and these in turn appear in the developed organisms. This argument and his emphasis on experiment as the means of settling such questions were recalled by PROFESSOR CONKLIN thirty-eight years after (1934) in a similar symposium before the American Philosophical Society on the subject "Fifty Years of Progress in Evolution" with justifiable pride.

All of CONKLIN's books were initiated in one or more public lectures which were frequently requested because of his clear and lucid presentations of biological problems. The last of these was "Man: Real and Ideal" (1943). Here facts of development and heredity are summarized, and the author goes on to discuss in some detail what he believed to be the bearing of these biological data on philosophical systems, such as realism and idealism, and problems of individual and social responsibility. Although CONKLIN's public presentations of these problems were always received with interested discussion, his later books were less successful than his earlier less pretentious summaries of the scientific facts. Although PROFESSOR CONKLIN continued his laboratory studies almost to his final years, he always found time and place to keep informed on

the work of younger colleagues working on problems in animal development or genetics. His quick, retentive mind seldom failed to illumine a discussion with suggestions based on his knowledge of earlier experiments. Throughout his long life he continued to emphasize that the methods and discoveries of genetics have been the most fruitful additions to biological science of the past century and give the experimental proof for many of the generalizations of DARWIN and WEISMANN.

HAROLD H. PLOUGH

PARTHENOGENESIS IN DROSOPHILA

HARRISON D. STALKER[1]

Department of Zoology, Washington University, St. Louis 5, Missouri

Received April 27, 1953

PARTHENOGENESIS, although well known in the lower Diptera, e.g., Miastor, has been rather infrequently reported, and apparently never analyzed, in the Acalypteratae. Thus the discovery of facultative parthenogenesis in several species of the genus Drosophila (STALKER 1951, 1952) seems of sufficient interest to report in some detail, especially as members of this genus have been so much studied in regard to their genetics and evolution.

Previous records of Acalypterate parthenogenesis involve the two families Octhiphilidae (STURTEVANT 1923), and Agromyzidae (HERING 1926; FRICK 1951). In addition STURTEVANT (1923) has given evidence for parthenogenesis in the family Lonchopteridae (Brachycera). Many additional cases of parthenogenesis in other orders of insects are cited by SUOMALAINEN (1950) in his general review of parthenogenesis in animals.

MATERIALS AND METHODS

Most of the work to be reported in this paper was done with strains of *Drosophila parthenogenetica* STALKER originally derived from a single strain established by the University of Texas workers from a collection made in Atlixco, Mexico (PATTERSON and MAINLAND 1944) and generously supplied by the Texas laboratory. The specimens of *Drosophila polymorpha* used were derived from a Brazilian strain supplied through the kindness of DR. TH. DOBZHANSKY. Both of the above forms belong to the *D. cardini* species group.

In the measurements of rates of parthenogenesis reported in this paper, the virgin females were kept, either singly or in groups, in vials with standard cornmeal-Karo-agar-Moldex food medium. All work was done at 25°C unless otherwise indicated. The virgin females were transferred to fresh food vials every day, or in some cases every second day. The vials from which the females had been transferred were examined under a dissecting microscope daily up to and including the fourth day after oviposition. In these examinations the numbers of dead embryos and inviable larvae, the numbers of viable larvae, and the numbers of apparently undeveloped eggs were recorded.

The dead embryos were detected by the discoloration associated with their decomposition. When the typically mottled-looking brownish-black eggs were discovered they were in most cases removed, cleaned of food, crushed under a coverslip in saline and examined under high power with a compound micro-

[1] These studies were aided by a contract between the Office of Naval Research, Department of the Navy and Washington University (NR 164 10). Additional support was received from the Rockefeller Foundation and Washington University.

Second Printing 1966 / University of Texas Printing Division, Austin

scope. In only one case out of over a thousand brownish eggs so examined was there no indication of any larval structure such as mouth hooks, gut lining, tracheal tubes or segmental hooklets. This method of detecting early embryonic development would of course result in the missing of those cases in which embryonic development was terminated very early, or in which no discoloration occurred, and should thus give an underestimate rather than an overestimate of the frequency of embryonic development.

When impaternate larvae were detected they were classified as viable or inviable on the basis of their appearance, this classification being checked in later examinations, and corrected if necessary. With a little experience there was no difficulty in making an accurate classification at the time the larvae were first detected. The inviable larvae typically showed enlarged mouth hooks and/or anterior region, and did very little active feeding; they displayed a great deal of slow wandering around, often leaving the choicest food (the yeast). The viable larvae on the other hand fed actively and almost constantly, and tended to remain in the region of the yeast droplet.

The mature (viable) larvae of both *D. parthenogenetica* and *D. polymorpha* have the distressing habit of leaving the food and pupating in the cotton plug of the vial, where they generally dry out. This difficulty may be partially overcome by the use of secondary plugs of cleansing tissue inserted in the vial well below the cotton plug. However, even the use of such a secondary plug will usually not save all of the larvae from self-destruction, and for this reason a count of viable larvae usually gives a better indication of fertility in vials than does the number of adults finally produced.

THE PROBLEM

Parthenogenesis in Drosophila was first discovered in a strain of *D. parthenogenetica,* which at that time was incorrectly classified as *D. neocardini* and has since been described (STALKER 1953). Crosses between *D. parthenogenetica* females and males of other species of the *D. cardini* species group resulted in the production of daughters which were morphologically, chromosomally and physiologically identical with their mothers. Further tests with virgin *D. parthenogenetica* females showed that parthenogenesis rather than hybridization was involved.

A number of questions then arose: 1. Did the production of diploid progeny by diploid virgins involve ameiotic chromosomal behavior with no chromosome reduction, or was there some type of meiosis with reconstitution of the diploid chromosome complement? 2. Although the percentage of unfertilized eggs completing development was less than 1.0, was it possible that intraspecific or interspecific matings might markedly increase this percentage? 3. Was it possible that suitable environmental conditions might bring about an increase in the frequency of parthenogenetic development? 4. Would continued selection for parthenogenetic development bring about an appreciable increase in its frequency? 5. Was facultative parthenogenetic development really a widespread phenomenon in Drosophila which had been hitherto over-

looked because of the low percentage frequency of development of unfertilized eggs? 6. What is the adaptive value, if any, of facultative diploid parthenogenesis in Drosophila? It was in an attempt to answer these questions that the experiments reported below were conducted.

Any effort to determine the chromosomal behavior in unfertilized eggs had, for practical reasons, to be directed along genetical lines, since direct cytological study of the unfertilized eggs was impractical due to the small number actually completing normal development. Thus at the outset efforts were made to obtain at least one mutant gene of which the transmission could be studied as an indication of chromosomal behavior. X-ray treatment of *D. parthenogenetica* females with 4000r units was followed by the discovery of a sex-linked recessive mutant gene, *g*, " garnet." Garnet flies are easily separable from wild-type, show rapid development and are little reduced in viability.

THE PROGENY OF DIPLOID VIRGINS

Table 1 shows the progeny of a selected strain of diploid females which have now been maintained without males for thirty-one consecutive generations. This strain was carried in vials for the first seventeen generations, and during this period nearly all of the diploid females produced in each generation were

TABLE 1

Progeny of diploid virgins of D. parthenogenetica; unisexual strain, generations 6 through 14. Progeny reared in vials.

Generation	Total progeny	% Diploid females	% Triploid females	% Diploid males	% 2n-3n Mosaic females
6	62	62.90	32.26	1.61	3.23
7	339	73.75	25.66	0.59	0.00
8	486	72.84	25.72	1.23	0.00
9	622	70.58	27.17	1.77	0.48
10	421	76.01	20.43	2.14	1.19
11	734	72.48	24.80	1.63	0.95
12	744	77.96	20.56	0.67	0.81
13	548	73.54	23.18	2.74	0.36
14	1182	78.34	20.05	1.43	0.17
Totals	5138	74.80*	23.10**	1.50***	0.53****

*Of the diploid females, 0.39% showed abnormal abdomen, 0.03% were 5-legged, and 0.03% were Minute.

**Of the triploid females, 6.82% showed abnormal abdomen, 1.18% were 5-legged, and 0.08% were 7-legged.

***Of the diploid males, 1.30% were 5-legged.

****Two diploid-triploid mosaics were found which were apparently diploid-male, triploid-female sex-mosaics. In addition to the types listed in the table, three polyploid intersexes were found which, on the basis of the wing-cell size were considered to be 2X 3A intersexes.

used as the parents of the next. Since the seventeenth generation the strain has been carried in standard half-pint culture bottles, with about 400 diploid females used as parents in each generation. The data in the table cover the progeny produced in generations six through fourteen. It will be noted from this table that for the nine generations shown there is very little change in the frequency of the four types of progeny. About 1.5% of the progeny are diploid males. These males are sterile, as expected, and cytological analysis of the testes has shown them to be XO rather than XY. The diploid and triploid females are readily distinguished by the spacing and size of the hairs on the wing blade. Since each hair is derived from a single cell, a count of the number of hairs per unit space gives the cell size and hence indicates whether diploidy or polyploidy is present (DOBZHANSKY 1929). Cytological examination of the ovaries of eleven females which, by their wings were clearly polyploid established that they were *triploid,* not tetraploid. Using the cell size of the wings in these proven triploid females as a guide, counts were made of the wings of forty-two more polyploid females, and these agreed so well with the cell size of the original eleven females that there was no doubt that they too were triploid. No clearly tetraploid females have been found so far among the progeny of diploid virgins.

In order to establish the fact that triploidy in this species is definitely associated with parthenogenesis rather than an independent characteristic of the strain, twenty-three females from the eighth generation of the unisexual strain were mated to males of the unselected bisexual strain, the larvae reared in uncrowded bottles and the emergent adults checked for polyploidy by examination of the wings. Out of the 2,736 females and 2,182 males so checked all were diploid. Thus the diploid females of the unisexual strain regularly produce triploid progeny only from unfertilized eggs.

Approximately one-half of one percent of the progeny of diploid virgins were clearly diploid-polyploid mosaics. In one of these mosaic females one ovary was shown cytologically to be triploid, the other diploid. Comparison of the wings of the other mosaic individuals with those of cytologically proven triploids showed that the polyploid patches were clearly triploid, not tetraploid. The *observed* frequency of such mosaics is probably lower than the frequency with which they are produced, since although a mosaic in which one wing is diploid and the other triploid could scarcely be overlooked, in some cases one wing was either diploid or triploid, and the other mosaic, and some of these latter cases were undoubtedly missed.

As indicated in the footnotes to table 1, abnormal abdomen and the absence of one of the metathoracic legs occurred in both diploid and triploid females, but was much commoner in the latter. The seven-legged triploid female showed a complete duplication (mirror-image) of one metathoracic leg, the duplication beginning with the coxa.

In each generation of the unisexual diploid strain, starting with the progeny of generation six, the triploid virgins were separated and allowed to reproduce. Table 2 shows the progeny so produced.

TABLE 2

Progeny of triploid virgins of D. parthenogenetica; unisexual strain, generations 7 through 15. In each generation the triploid mothers were derived from diploids. Thus each generation of progeny shown in this table are second generation derivatives of the unisexual diploid line. Progeny reared in vials.

Generation	Total progeny	% Diploid females	% Triploid females	% Diploid males	% 2n–3n Mosaic females
7–8	174	25.86	27.01	47.13	0.00
9–11	300	27.67	29.67	41.67	0.33
12	343	32.65	25.07	41.69	0.29
13	293	32.42	29.35	37.54	0.68
14	262	22.14	37.02	39.31	0.38
15	428	29.44	29.91	39.02	0.70
Total	1800	28.83*	29.61#	40.56**	0.44***

*Of the diploid females, 1.16% showed abnormal abdomen, 0.77% were Minute and 0.19% were 5-legged.

#Of the triploid females, 3.94% showed abnormal abdomen and 1.69% were 5-legged.

**Of the diploid males, 5.21% were Minute.

***Two diploid-triploid mosaics were found which were apparently diploid-male, triploid-female sex mosaics.

It will be noted from this table that the frequencies of the various types of progeny of triploid virgins are strikingly different from those derived from diploids. This difference involves principally a change in the frequencies of diploid XO males and diploid females. While diploid males are rarely produced by diploid virgins, they constitute over 40% of the progeny of triploids. These diploid males were also XO and sterile. The diploid and triploid progeny showed the same disparity in the frequency of abnormal abdomen and the five-legged condition as that shown for the diploids and triploids in table 1.

Table 3 lists the progeny obtained from virgin diploid females heterozy-

TABLE 3

Progeny of heterozygous garnet (+/g) virgin females obtained by crosses of homozygous wild-type females × garnet males.

	Diploid males	Diploid females	Triploid females	2n–3n Mosaic females
Wild-type	6	1,284*	172**	5
Garnet	7	279	0	1

*42 of these diploid wild-type females were analyzed by crosses to fertile garnet males. Of these 42, 10 were homozygous wild type, 32 were heterozygous for garnet. Thus the corrected estimates for the three genotypes would be: *g/g* 17.9%; +/*g* 62.6%; +/+ 19.6%.

**65 triploid wild-type females were analyzed by crosses to fertile garnet males. Of these, 64/65 were either +/+/*g* or +/*g*/*g*. 1/65 may have been +/+/+. Such a +/+/+ triploid female could have arisen from an homozygous wild-type impaternate virgin female.

gous for the sex-linked recessive eye-color, garnet (g). It will be noted that among the diploid female progeny, heterozygotes and both types of homozygotes appear, with about 63% heterozygotes and 37% homozygotes. Among the triploid female progeny no homozygous garnet individuals appeared. On the basis of these data alone it might be supposed that perhaps the absence of garnet triploid females among the progeny was due to their reduced viability. However, garnet triploids have been produced in quantity in other crosses and show about 80% viability as compared to wild-type triploids. Of the 172 triploid females produced, 65 were tested by crossing them to garnet males, and 64/65 were shown to carry at least one garnet gene, and thus were either $+/g/g$ or $+/+/g$. The one triploid female which when tested gave no indication that it carried a garnet gene, and was thus $+/+/+$, may have arisen from an impaternate homozygous wild-type diploid mother, since the heterozygous garnet diploid virgins used as parents were produced by crosses of homozygous wild-type females × garnet males, and any unfertilized eggs which completed development could have produced homozygous wild-type diploid females. The production of $+/g$ virgins by the reciprocal cross was not practical since no bisexual strain showing a sufficiently high rate of parthenogenesis was available.

The above data give some very important clues regarding the meiotic mechanism in unfertilized eggs, and before presenting more data a partial analysis will be made.

To begin with, it is clear that in the production of progeny from unfertilized eggs in *D. parthenogenetica,* some meiotic mechanism is involved, rather than an ameiotic maturation of the egg in which there is no chromosome reduction and hence no need for reconstitution. This conclusion is based on several facts: First, diploid virgins produce not only diploid daughters like themselves, but triploid daughters as well. If the egg maturation were ameiotic, only diploids (and possibly tetraploids) should be produced. Secondly, heterozygous garnet diploid virgins produce not only heterozygous daughters like themselves, but large numbers of both types of homozygotes. Thus genetic recombination is occurring, requiring some sort of meiotic phenomenon.

In those forms in which partheno-produced individuals have a zygoid (diploid or polyploid) number of chromosomes, SUOMALAINEN (1950) distinguished two general kinds of parthenogenetic mechanisms, as follows:

"1. Automictic parthenogenesis or parthenogamy. Regular chromosome conjugation and reduction occur in the eggs developing parthenogenetically. The zygoid chromosome number is restored through the fusion of two azygoid nuclei, the formation of a restitution nucleus or endomitosis. This corresponds to WHITE'S (1945) meiotic parthenogenesis.

"2. Apomictic parthenogenesis. Neither chromosome reduction nor fusion of nuclei nor any corresponding phenomenon . . . takes place in the eggs developing parthenogetically. This type corresponds to the somatic parthenogenesis of WINKLER and ANKEL and the ameiotic parthenogenesis of WHITE (1945)."

It appears then that *D. parthenogenetica,* having occasional diploid parthenogenesis associated with a meiotic mechanism would be classified as a facultative automict.

The various types of automictic parthenogenesis are reviewed by SUOMALAINEN (1950) and will be considered here only insofar as they are directly concerned with the argument.

In some automictic forms (e.g., the Psychid moth, *Apterona helix*) the first meiotic division proceeds normally, but both second division metaphase plates fuse to form a single metaphase plate, the second division then resulting in the formation of two diploid nuclei, both of which function as cleavage nuclei. In this type of parthenogenesis it will be noted that there is no formation of nonfunctional polar nuclei, and hence no elimination of genes between the onset of meiosis and the first cleavage divisions. Thus a female heterozygous for garnet could not produce either homozygous garnet or homozygous wild-type progeny. Moreover if there were post-reduction of the garnet gene in this type of meiosis (and this might be expected, at least part of the time), then the two diploid nuclei formed at the end of meiosis could be homozygous garnet in the one case and homozygous for the wild allele in the other, and a garnet mosaic should be formed. Actually among the 1,563 diploid daughters of heterozygous garnet virgins no mosaics for garnet have been found. Finally, the type of meiosis found in *Apterona helix* would not, without special modifications, account for the observed production of 23% triploid females from diploid virgins. Thus it is clear that any meiotic mechanism of this type simply cannot explain the facts.

Another type of automictic parthenogenesis (e.g., in the gall wasp, *Neuroterus baccarum*) involves an abortive first meiotic division with the result that only one nucleus is formed rather than two, followed by a second division resulting in the formation of two nuclei, both diploid and both functioning as cleavage nuclei. Genetically the results of such meiotic behavior in heterozygous garnet diploid females would be the production of either heterozygous or garnet-mosaic diploids. Thus for the reasons outlined above in relation to *Apterona helix* this type of meiotic behavior cannot explain the known facts.

A variation of the meiotic behavior of the two forms described above involves an abnormal first meiotic division, followed by a second division and the formation of two diploid nuclei, but with only one of these nuclei functioning as the cleavage nucleus, the other being eliminated as the polar nucleus (e.g., the moth *Solenobia lichenella* and the parasitic wasp *Nemeritis canescens*). Genetically the results of this chromosomal behavior would be that an heterozygous garnet female could produce either homozygotes or heterozygotes, the proportions depending on the amount of crossing over between the locus of garnet and the X-chromosome centromere, i.e., on the relative frequencies of pre- and post-reduction of garnet or its wild-allele. However, this meiotic mechanism will still not explain the high production of triploids by diploid virgins.

All of the types of meiosis outlined above have resulted in the formation of diploid cleavage nuclei because of abnormalities in the meiotic mechanism itself. However even with normal meiotic divisions diploidy may be reconstituted if the haploid cleavage nuclei fuse in pairs, forming diploid nuclei

(e.g., as in the moth *Solenobia triquetrella*). Such fusion of the cleavage nuclei could, if it involved three haploid cleavage nuclei rather than only two, result in the formation of triploids as well as diploids. However, an heterozygous garnet diploid virgin should then always produce homozygous progeny, since all of the cleavage nuclei, whether they fused in two's to form diploids, or three's to form triploids would all come from the same original haploid egg pronucleus. The fact that the diploid progeny of heterozygous diploid virgins are either heterozygous or homozygous, and the fact that the triploid progeny of heterozygous diploid virgins are never homozygous garnet nor (probably) homozygous for its wild allele, both effectively eliminate the possibility that this type of chromosomal behavior could occur regularly in *D. parthenogenetica.*

One final way in which meiosis might be perfectly normal, with the formation of an haploid egg pronucleus and three haploid polar nuclei, involves a fusion of either the egg pronucleus with one of the polar nuclei, forming a diploid first-cleavage nucleus, (e.g., as in the coccid *Lecanium hesperidum*), or possibly the fusion of two polar nuclei (the egg pronucleus and one polar nucleus being eliminated in cleavage). Similarly, fusion of three of the four haploid nuclei would result in the production of a triploid first-cleavage nucleus.

It will be noted that of all these types of meiosis which are known to occur in various parthenogenetic animals, only one type, the last, could, with some modifications, account for the findings in *D. parthenogenetica.*

We will provisionally assume that this last type of meiotic behavior is the one occurring in *D. parthenogenetica,* and consider in some detail how well the available data fit such an assumption.

First, let us consider the commonest types of progeny of the diploid virgins. These are: diploid females and triploid females. It will be noted from figure 1-a that fusion of any two of the haploid nuclear products of the second division will result in a diploid first-cleavage nucleus, while fusion of three haploid nuclei would result in a formation of triploids. Although cytological data on normal meiosis in *D. parthenogenetica* are lacking, studies of other Diptera, especially Drosophila indicate that such fusion of the haploid second division products is the rule in normal meiosis of *fertilized* eggs. Thus, in Sciara, CARSON (1946) finds fusion of the two median nuclei to form a diploid nucleus. This diploid nucleus and the terminal nucleus (one of the first polar bodies) then migrate to the posterior end of the egg and do not take part in further development. In *D. melanogaster,* HUETTNER (1924) has shown that following fertilization and the completion of meiosis there is fusion of the two inner polar nuclei to form a diploid nucleus, followed by a fusion of this nucleus with the remaining polar nucleus to form a triploid nucleus, the triploid nucleus so formed from the three polar nuclei eventually disintegrating. FAHMY (1952) has studied meiosis in fertilized eggs of *D. subobscura,* in which he finds that following the second meiotic division there is regularly fusion of the two inner polar nuclei to form a diploid nucleus, and that this is followed by fusion with the remaining polar nucleus to form a triploid nucleus which persists without

further division into late cleavage. Thus it would appear that the normally occurring fusion of haploid polar nuclei in fertilized eggs of Drosophila, and probably many other Diptera as well, furnishes a ready-made mechanism which might under certain circumstances " take over " in the development of unfertilized eggs, and result in the production of the diploids and triploids so common among the progeny of virgin *D. parthenogenetica.*

It will be noted that about 1.5% of the progeny of diploid virgins are XO sterile diploid males. Such males would require some irregularity in the meiotic mechanism, and it is suggested that non-disjunction of the X-chromosome in the second meiotic division would give such a result if, as indicated in figure 1-b fusion were between the two median polar nuclei. Such non-disjunction might also result in the production of triploid intersexes (fig. 1-b).

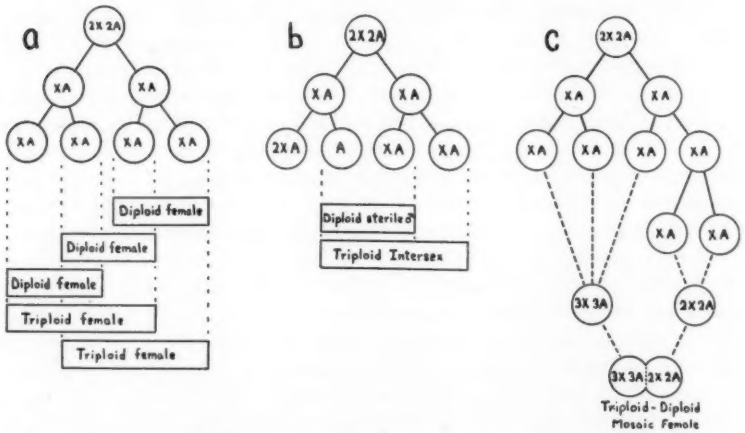


FIGURE 1.—Types of progeny which may be produced by various patterns of nuclear fusions in the eggs of diploid virgin females. In each diagram the four circles in the third row represent the four nuclei formed after the second meiotic division. See text.

Individuals which were clearly sub-triploid on the basis of wing-hair counts, and were intersexual in their internal genitalia have been observed. If, instead of the terminal nucleus containing two X chromosomes, one of the median nuclei did so, then a median fusion of two polar nuclei would result in the production of 3X2A superfemales. A few females have been observed which on the basis of morphology may have been superfemales but no satisfactory chromosome counts have ever been obtained from their ovaries.

In the case of the 0.5% diploid-triploid mosaics produced by diploid virgins a somewhat more complicated explanation is required. Probably the simplest is that shown in figure 1-c. This involves mitotic division of one of the four haploid nuclei prior to fusion, fusion of two of the haploid nuclei so produced to form a diploid nucleus, and this diploid nucleus plus a triploid nucleus formed by a simple three-way fusion both functioning as cleavage nuclei, thus forming a diploid-triploid mosaic.

If this is the correct explanation, then the diploid tissue might be +/+ or *g/g* while the triploid tissue would always be phenotypically wild-type (+/+/*g* or +/*g*/*g*). Thus some garnet diploid-wild-type triploid mosaics might occur. Only six ploid mosaics have been produced by +/*g* diploid virgins and none of these was mosaic for eye-color.

It will be noted from table 2 that triploid virgins produce a very high proportion of triploid and diploid daughters. In figure 2 are shown only those types of triploid meiosis involving segregation of complete haploid sets of autosomes. It will be noted that regardless of the relative segregation of autosomes and X-chromosomes, a two-nucleus median fusion will always result in the formation of a triploid cleavage nucleus. This is true even if the autosomes do not assort as complete haploid sets. Figure 2-a shows two ways in which diploid females might be produced; either by fusion of two haploid

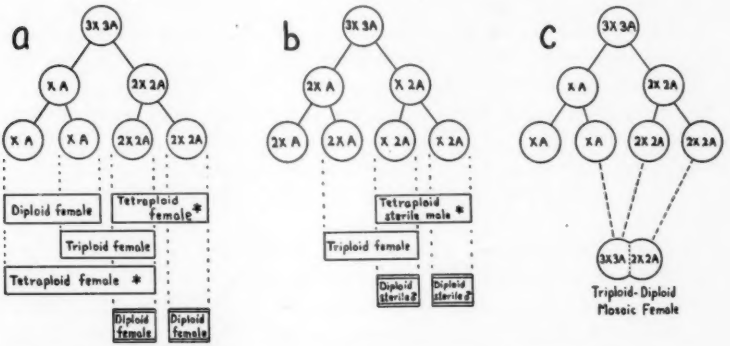


FIGURE 2.—Types of progeny which may be produced by various patterns of nuclear fusions in the eggs of triploid virgin females. In each diagram the four circles in the third row represent the four nuclei formed after the second meiotic division. Types of progeny marked with an asterisk have not been observed. See text.

nuclei, or by direct development without fusion of one of the two remaining diploid nuclei.

The most frequent type of progeny of the triploid virgins is the diploid XO sterile male. In the absence of extraordinary assumptions concerning nondisjunction of X-chromosomes or autosomes, this large number of diploid males could only be produced by direct development, without fusion, from the X2A nuclei, as shown in figure 2-b. This apparent necessity for development without fusion to account for the production of diploid males, makes it highly probable that a certain proportion of diploid females are produced in the same way, as shown in figure 2-a. Whether in such cases the individuals so produced are actually mosaics, with both diploid nuclei functioning in cleavage, or whether one nucleus functions, the other taking no part in development, is not known.

Theoretically this point could be settled by a study of the progeny of +/+/*g* or +/*g*/*g* triploid females. With post-reduction of the garnet gene, diploid

males and females might be garnet mosaics if they developed from two unfused diploid nuclei rather than one. Unfortunately all of the several hundred triploid females that have been of the required genotype have proved to be so sterile as virgins that only 24 diploid progeny have been produced by them altogether. Since none of these progeny was garnet mosaic, the question remains unanswered, especially so since it is not possible to predict with any accuracy the relative frequencies of pre- and post-reduction of the garnet gene. At least it may be said that at present there is no evidence for such mosaic origin from unfused nuclei.

It is worth mentioning at this point that BRIDGES (see BRIDGES and ANDERSON 1925) has shown that in triploid females of *D. melanogaster,* meiosis of the type shown in figure 2-b of this paper is much commoner than the alternate type 2-a. If this finding applies also to *D. parthenogenetica* it may well explain the relatively high proportion of diploid males to diploid females among the progeny of triploid virgins.

Fusions of the types shown in figures 2-a and 2-b should result in the formation of both tetraploid males and females. Such males and females have not been found, which suggests that they are low in viability or very rare.

The triploid-diploid mosaic females which make up about one-half of one percent of the progeny of triploid virgins may be accounted for by three of the four nuclei taking part in cleavage, but the two median nuclei forming a triploid cleavage nucleus, the outer diploid nucleus cleaving without fusion, as in figure 2-c.

The three diploid-triploid sex-mosaics (listed in the footnote of table 2) could be produced by the same type of chromosomal behavior, starting with the X-autosomal distribution of the type shown in figure 2-b. Here fusion of the two median nuclei would provide the triploid cleavage nucleus, the outer X2A unfused nucleus would supply the male cleavage nucleus, thus leading to a female-triploid male-diploid mosaic.

THE FUSION PATTERN IN DIPLOIDS

The evidence given above indicates that in the production of diploid daughters by diploid virgins two of the four haploid second division products fuse, to form the diploid first-cleavage nucleus. Which particular nuclei take part in such fusion is of considerable interest. In Drosophila the termination of the second division leaves the nuclei temporarily in a straight line extending perpendicularly from the periphery of the egg, and there is indirect evidence that at least some of the two-nucleus fusions must involve the two median nuclei. This is based on the fact that in triploid virgins a high proportion of the progeny are triploid daughters. Such daughters would always be formed by fusion of the two median nuclei, regardless of how the sex chromosomes and autosomes segregated in the first division, and apparently there is no other way in which triploids could be formed from triploid mothers (see fig. 2-a, 2-b). Thus, in the eggs from triploid females at least, the evidence is very strong that median fusions of the second division products do

occur. This still leaves the question of terminal fusions, such as might occur between the egg pronucleus and the nearest polar nucleus, or between two terminal polar nuclei. Of course in the formation of triploids from diploids (fig. 2-a), it is presumed that both median and terminal fusions occur, but otherwise there is no class of offspring which has been observed from either diploid or triploid virgins which would *require* terminal fusion alone. Which particular nuclei take part in fusions might be determined genetically by following the transmission of a gene located so close to a centromere that there was little or no crossing-over. In the absence of such a gene, some information may be obtained by the behavior of the Y chromosome in XXY females. Such females were obtained by crossing triploid garnet females to wild-type males. The resulting diploid garnet daughters should, unless they were impaternate, then receive both X-chromosomes from the mother and a Y-chromosome from the father. A number of such females were allowed to reproduce as virgins, but for reasons imperfectly understood only two of them produced enough

TABLE 4

The chromosomally different types of progeny produced by virgin XXY diploid females.

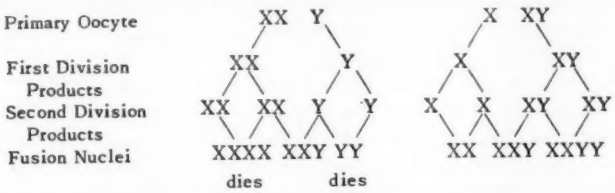
Chromosomal constitution	No.	Fusion pattern*
Diploid XX	3	Terminal
Diploid XX-YY	2	Terminal
Diploid XX-Y	12	Median
Triploid XXX-Y	4	
Triploid XXX-YY	2	

*See text for explanation.

progeny to indicate with certainty that they were XXY, and not XX impaternates. The metaphase chromosomes of the impaternate progeny of these two females were studied by making acetic-orcein smears of the larval neuroblasts. In the metaphase chromosomes of *D. parthenogenetica* the X-chromosome is a long rod with a satellite. The Y is a large, approximately equal-armed V-shaped chromosome, the autosomes consist of a large pair and a small pair of V-shaped chromosomes and a pair of dots. The distinctive size and shape of the sex-chromosomes thus makes analysis of the metaphase plates relatively easy. Table 4 lists the chromosomally different types of progeny produced. Since the triploid XXX-Y and XXX-YY females are derived from combined median and terminal fusions they are of no value in determining the fusion pattern and will not be considered further.

In interpreting the diploid progeny an assumption must be made. The assumption is that in XXY females the sex chromosomes show pre-reduction, as they would be expected to do in an XY diploid male. There appears to be no good reason for believing that there should *not* be pre-reduction, but with the genetic material at hand in this species this point cannot be readily settled.

With pre-reduction of the sex chromosomes the first and second divisions might proceed along either of the paths indicated below:



It will be noted that regardless of the pattern of non-disjunction, fusion of the two median nuclei will always result in XXY females, while fusion of two terminal nuclei will result in death (XXXX and YY classes), or in XX or XXYY individuals. Thus all of the living diploid females produced may be classified as having arisen from median or terminal nuclear fusions. The data in table 4 indicate that the fusions may arise by either path, 12/17 of the cases coming from median fusions, 5/17 cases coming from terminal fusions. Since approximately one-half of the terminal fusions should lead to the production of inviable karyotypes, the limited data available would suggest that median and terminal fusions are about equally frequent.

PARTHENOGENETIC DEVELOPMENT IN OTHER DROSOPHILIDAE

The finding of parthenogenesis in *D. parthenogenetica* has drawn attention to the possibility that such unusual reproductive behavior might occur in other related species, or for that matter in any other Drosophiline. Even in genetically well-investigated species rare parthenogenetic development might be overlooked, especially if it regularly terminated with death in embryonic or early larval life.

In order to investigate this possibility, virgin females of twenty-seven additional species of Drosophilidae were maintained throughout life in vials without males, the eggs they produced being examined under a dissecting microscope every day, or in some instances every other day up to and including the fourth day after oviposition. By this procedure it was possible not only to identify impaternate larvae when they were produced, but also in many instances to observe embryos which had died prior to hatching.

In those cases where larvae appeared they were at once transferred to fresh food along with some of the yeast in which they were found, and retransferred as often as necessary to keep down mold and bacterial growth.

The results of this survey are summarized in table 5. The total numbers of eggs examined (listed in the second column), are approximations based on actual counts of the numbers of eggs laid in every third vial through which the females were transferred. In the case of the figure " 500,000 " for *D. melanogaster,* this is an approximation based on the known egg production per female under crowded conditions in vials, multiplied by the number of females tested. It is probably an underestimate.

TABLE 5

Summary of rates of parthenogenetic development in various species of Drosophilidae. Rates for D. parthenogenetica are not included in this table. Members of the same species group are bracketed.

Species	Total unfertilized eggs examined	Total dead embryos	Total dead larvae	Adults
DROSOPHILA				
cardini #	52,850	3		
polymorpha #*	37,629	109		2
neocardini #	11,439	1		
cardinoides #	30,777	3		
acutilabella #	16,463	11		
campestris #	23,682	14		
acutilabella X cardinoides F_1 hybrids #	6,231	1		
macrospina	2,655			
funebris	43,198	3		
melanica	5,199	1	1	
nigromelanica	5,243			
americana	18,165			
tripunctata	9,280	2		
mediostriata #	14,883	7		
robusta	10,706	2		
immigrans	8,153	1		
transversa	11,616	2		
quinaria	11,868	6		
putrida	8,431	4		
hydei	63,027	18	1	
HIRTODROSOPHILA				
duncani	16,044	1		
SOPHOPHORA				
melanogaster	32,197	1		
melanogaster**	500,000		2	
simulans	13,872	1		
affinis	19,059	4		1
PHOLADORIS				
victoria	1,292			
SCAPTOMYZA				
graminum	4,314	4		
adusta	2,340			
ZAPRIONUS				
vittiger #	10,167			

#Virgins from established laboratory stocks. In all other cases the virgins were F_1 or F_2 progeny of wild flies captured in the St. Louis, Missouri area.

*These figures are for the unselected bisexual strain. See table 8 also.

**Approximate number of eggs, oviposition in half-pint bottles, unfertilized eggs not examined for presence of dead embryos.

The first seven rows in the body of table 5 show the results for the other members of the species group to which *D. parthenogenetica* belongs (the *D. cardini* group). It will be noted that in each of the six species and in the hybrids some dead embryos were found. However, in only one species, *D. polymorpha,* were any adults produced, and in this case only two (diploid females), from about 37,000 eggs. These results for *D. polymorpha* are based on the performance of the unselected, bisexual Brazilian stock received from DR. TH. DOBZHANSKY. A selected strain derived from one of the two impaternate females shows a much higher rate of parthenogenesis, and is discussed later in the paper.

For the remaining species belonging to the subgenus Drosophila, ten out of the thirteen studied showed some parthenogenetic development terminated prior to hatching, and two species, *D. melanica* and *D. hydei* each produced a single impaternate larva which survived into the second larval instar.

In the subgenera Hirtodrosophila and Sophophora all four of the species tested gave positive results. It will be noted that in the case of *D. affinis* one impaternate adult was produced, which unfortunately was lost before it could be sexed. However, there was little doubt that the pupa from which this fly came belonged to the *D. affinis* group, as indicated by the structure of the pupa case and the coloration of the developing imago. The possibility that this individual was the result of contamination seems very remote as no stock of *D. affinis* or its relatives had been maintained in the laboratory for a period of six weeks prior to the emergence of this fly.

It will be noted that both *D. melanogaster* and *D. simulans* produced impaternate embryos. In the case of *D. melanogaster* the second entry in the table refers to a special test made of the first generation daughters of many wild caught flies. In this case none of the eggs were examined microscopically, although the bottles were frequently examined for the presence of larvae two of which were detected but which did not survive beyond the second instar.

Approximately half of these 500,000 eggs came from females mated to sterile males produced by the use of MULLER's " sterilizer " stock, the others were produced by virgin females. These tests with *D. melanogaster* seem to indicate that, as has been concluded in the past, parthenogenesis in this species does not constitute a serious complication in genetic experimentation.

In the genera Scaptomyza and Zaprionus only one of the three species tested (*S. graminum*) showed parthenogenetic development. Thus of the twenty-seven species examined, two produced impaternate adults, three produced second instar larvae, fifteen produced only dead embryos, and five showed no indication of parthenogenetic development of any sort.

In no case was it possible to determine the chromosomal content of the developing embryos, since they were only discovered after they had died and decayed. In the case of the larvae dying in the second instar the condition of the tissue likewise prohibited cytological analysis. However, from the evidence given above in connection with *D. parthenogenetica,* it is assumed that in this case the death in the embryos and partly grown larvae was associated with haploidy.

FACTORS AFFECTING RATES OF PARTHENOGENESIS

1. *The genotype and the karyotype*

As explained above, in the species *D. parthenogenetica* the unisexual parthenogenetic strain was established in the summer of 1951 and has been maintained since that time, now being in its thirtieth generation. Of course in such a strain, with complete dependence on parthenogenetic reproduction any combination of genes favoring such reproduction would have a selective advantage, and would tend to be incorporated into the strain. Thus the rates of parthenogenesis in such a strain might be expected to show a gradual rise. Table 6 shows the rates for the unselected bisexual strain and for the second, ninth and seventeenth generations of the unisexual strain. It will be noted that the strain has improved itself in two ways. First, the frequency of eggs starting development has risen from 0.91% to 8.20%. Secondly, the *survival* of individuals which are known to have *begun* development has risen from 8.96% to about 20%. These two factors taken together have increased the production of viable impaternate progeny from 0.08% to 1.55%, or almost twenty-fold. It will be noted in this and later tables that instead of considering the percentage of eggs forming adults as an indication of fertility, the percentage forming viable larvae is used.

When the females of the unisexual strain are outcrossed to unselected bisexual strain males the F_1 virgins appear to show a fertility intermediate between that of the two parental strains, although no numerical data are available to prove this.

Several points should be mentioned with regard to the unisexual strain. First, although 250 to 300 females are used as parents in each generation, it is not known how many of them actually produce progeny under the rather competitive conditions of the culture bottles, and hence the strain may, and

TABLE 6

Rates of parthenogenesis among diploid and triploid virgins of D. parthenogenetica.

Source of virgins	Diploid virgins				Triploid virgins
	Bisexual strain	Unisexual Selected Strain			Unisexual strain
		Generation 2	Generation 9	Generation 17	Generation 6 & 8
No. ♀♀ tested	218	82	69	42	54
Total eggs	74,538	20,751	17,740	12,464	11,234
Dead embryos	620	242	327	829	82
Larvae	61	64	66	193	19
% Eggs starting development	0.91	1.48	2.22	8.20	0.90
% Eggs forming viable larvae	0.08	0.31	0.37	1.55	0.17
% Survival among eggs starting development	8.96	20.92	16.79	18.88	18.81

probably does, consist of a group of very closely related individuals. Secondly, any genetic improvement in the reproductive performance of the strain due to recombination can only come from recombination of genetic variability *within* individuals, rather than *between* individuals, since there can be no mixing of the genotypes from any two individuals. Thirdly, the noted improvement in the fertility of the unisexual strain may be due to nuclear changes, cytoplasmic changes or both. The possible role of an infective or symbiotic agent as a promotor of parthenogenesis is under investigation.

The last column of table 6 shows the rates for triploid virgins derived from diploids of generations five and seven of the unisexual strain. Thus these triploids really represent the sixth and eighth generation of the strain, and may best be compared with the diploid virgins of the ninth generation. It will be noted that both the observed frequency of eggs known to start development and the percentage of eggs leading to the formation of viable larvae is lower than in the case of diploid females of the ninth generation.

The reasons for this are far from clear, however, since nothing is certainly known either of the frequencies of various types of chromosomal segregants, nor of the proportion of aneuploids which would develop far enough to be detected and classified as dead embryos.

The unisexual strain of D. polymorpha. Table 5 shows that in the unselected bisexual strain of *D. polymorpha* only two adults (diploid females) were produced. These two flies came from the same vial at the same time, and may well have come from the same mother. Of the two, one became accidentally stuck in the food; the other was tested as a virgin and of 693 unfertilized eggs 21.1% developed to the embryonic or early larval stage and died. Such a high proportion of eggs starting development has not been observed in any other case in this or any other species. However, despite the high proportion of eggs starting development, no viable larva were produced by this virgin. She was mated to normal XY ♂♂ from the unselected bisexual strain and a bisexual sub-strain established which was set aside for approximately a year. At the end of that period 52 virgin females from this sub-strain were tested and immediately produced an unisexual strain which has been maintained with relatively little difficulty since and is now in its ninth generation. Repeated attempts to establish a second unisexual strain from the original unselected bisexual strain have failed. This sequence of events seems to point to a rather abrupt genetic differentiation which caused a sudden rise in the rate of parthenogenesis. In *D. polymorpha* as in *D. parthenogenetica* both diploid and triploid females, as well as sterile XO males are produced by virgin diploid females, suggesting that the mechanism of parthenogenesis is the same in both species.

To summarize: It appears that both the genotype and the degree of ploidy may control intra-specific differences in the rate of parthenogenesis.

2. *The effect of female age on the rate of parthenogenesis*

Experience with the unisexual strain of *D. polymorpha* soon showed that the young virgins appeared to be less fertile than those two or three weeks

TABLE 7

Effect of female age on rate of parthenogenesis in D. parthenogenetica. The females reported in this table belonged to the 21st generation of the unisexual selected strain.

	Age of females in days						
	6–9	10–13	14–17	18–21	22–25	26–29	30–34
No. females tested	14	14	14	14	9	9	9
Total eggs	2,732	3,747	3,217	2,453	1,482	1,196	1,290
% Eggs starting development	7.28	8.14	5.38	6.77	8.43	5.18	8.29
% Eggs forming viable larvae	1.39	1.28	1.27	1.18	1.55	1.38	1.78
% Survival among eggs starting development	19.10	15.74	23.70	17.47	18.40	25.81	21.50

of age. This apparent effect of female age was accordingly tested in both *D. parthenogenetica* and *D. polymorpha.*

Table 7 gives the results for *D. parthenogenetica.* In this species fourteen virgins from the twenty-first unisexual generation were maintained separately in vials until they died. In the table the data from these fourteen females are lumped. Either considering the females as a group, or singly, they show no consistent change with age, either with regard to the percentage of eggs starting development, or for the percentage of eggs forming viable larvae.

In *D. polymorpha,* however (table 8), the proportion of eggs starting development and the proportion forming viable larvae rises until the third week of adult life; the increase in production of viable larvae being approximately twenty-fold. A consideration of the individual rates of the twenty females shows that there is a good deal of inter-female variability in the percentages of eggs producing viable larvae at different ages. In figure 3 the individual records are shown diagrammatically, with a single vertical bar for each female, the width of the bar indicating the percentage of eggs producing viable larvae

TABLE 8

Effect of female age on rate of parthenogenesis in D. polymorpha. The females reported in this table belonged to the 4th generation of the unisexual selected strain.

	Age of females in days						
	2–5	6–9	10–13	14–17	18–21	22–29	30–37
No. females tested	20	20	20	16	12	5	5
Total eggs	3,816	5,009	3,810	2,825	1,615	1,796	1,012
% Eggs starting development	0.26	1.04	2.05	3.89	5.57	3.29	0.49
% Eggs forming viable larvae	0.03	0.16	0.31	0.71	0.62	0.33	0.00
% Survival among eggs starting development	10.00	15.38	15.38	18.18	11.11	10.17	0.00

in a given age period. It will be noted from this figure that although there is much variability in life-length and fertility, very few young females produced any viable larvae, and for those that did, the percentage fertility was very low; while the older females were not only more frequently fertile, but showed a higher rate during their fertile periods.

The considerable inter-female variability in the viability and fertility of this unisexual strain of *D. polymorpha* may well be associated with strain heterozygosity for one or more deleterious genes, as approximately one-third of the females in the strain show abnormal drooping wings with the fifth longitudinal vein incomplete.

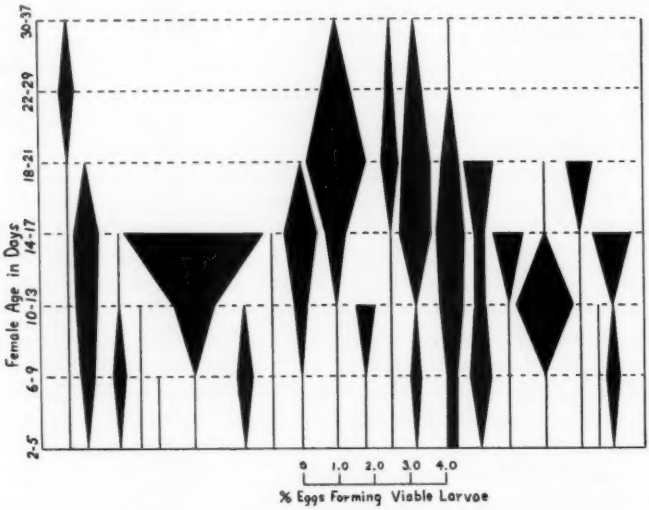


FIGURE 3.—Effect of age on rate of parthenogenesis in twenty *D. polymorpha* diploid virgins. The reproductive behavior of each female is represented by a vertical bar, with the width of the bar indicating the percentage of unfertilized eggs forming viable larvae at any given age. The height of the bar indicates the length of life for each female. It will be noted that in general the rate of parthenogenesis increases with age of female, and that also the proportion of females showing *any* parthenogenesis increases as the group ages. See also table 8 and text.

3. *Effect of temperature on parthenogenesis*

In an effort to find some sort of treatment which might increase the rate of parthenogenesis to a level at which the phenomenon could be studied more easily, many treatments and combinations of treatments, both of the virgin females and of the eggs which they produced were tried. Many of these preliminary experiments were poorly controlled, and if the experiments had had some slight effect on the rate of parthenogenesis, this effect could well have been overlooked. Some of the environmental factors tested were: type of

food, temperature of rearing and oviposition for the females, treatment of the eggs with ultra-violet, heat, cold and carbon dioxide. None of these, except high temperature, had any obvious effect. It was soon observed that holding the virgin females at 30°C for at least three days prior to oviposition resulted in a clear increase in rate of parthenogenetic development. The data on this point are summarized in table 9. The virgin females used in the controls and the three experiments were all reared at 25°C in the same set of culture bottles. In the column headed Expt. I are given the results of keeping females at 30°C for one week and then following the development of their eggs at the same temperature. It will be noted that the percentage of eggs starting development (including those which die in the embryonic or early

TABLE 9

Effects of temperature on rates of parthenogenesis in D. parthenogenetica. All females tested were reared at 25° C and were derived from the unselected bisexual strain. High temperature females were kept at 30° C for one week prior to collection of eggs.

	Controls: Females 25° C Eggs 25° C	Expt. I. Females 30° C Eggs 30° C	Expt. IIa* Females 30° C Eggs 25° C	Expt. IIb** Females 30° C Eggs 25° C
No. females tested	218	79	139	
Total eggs	74,538	22,025	42,729	5,286
Dead embryos	620	491	774	92
Larvae	61	18	60	14
% Eggs starting development	0.91	2.31	1.95	2.01
% Eggs forming viable larvae	0.08	0.08	0.14	0.26
% Survival among eggs starting development	8.96	3.54	7.19	13.21

*Females were kept at 30° C and changed to fresh food every 24 hours, at which time the vials with eggs were moved to the 25° C incubator.

**Females were kept at 30° C and changed to fresh food every 4 hours, at which time the vials with eggs were removed to the 25° C incubator.

larval stage as well as those which form viable larvae) is 2.31% in Expt. I as compared to 0.91% in the controls. This difference is highly significant, the chi square value of 272.7 leading to a P value of less than 0.001. However, in spite of the increase in the rate of eggs starting development, the percentage of developing eggs which survive to form viable larvae is much decreased, 3.54% for the experimentals as compared to 8.96% for the controls. This difference is also highly significant, the chi square value of 13.8 leading to a P value of less than 0.001. The net result of these two differences between the controls and Expt. I was that the percentage of eggs forming viable larvae remained the same: 0.08%.

It was felt that perhaps the higher mortality in the eggs starting development in Expt. I was at least partially a direct effect of temperature on viability. In order to test this Expts. IIa and IIb were carried out.

In Expt. IIa the females were maintained at 30°C as in Expt. I, but the vials containing the eggs were removed to a 25°C incubator every twenty-four hours, thus, the eggs remained at 30°C for a length of time which varied from 0 to 24 hours, and were kept at 25°C thereafter. In Expt: IIb a similar technique was employed, except that the freshly-laid eggs remained at the higher temperature for much shorter periods ranging from 0 to 4 hours.

In Expt. IIa the percentage of eggs starting development is significantly higher than in the controls, 1.95% as compared to 0.91%, but it is also significantly lower than in Expt. I, (chi square: 9.0; P value less than 0.003). In Expt. IIb also the percentage of eggs starting development is significantly higher than in the controls, 2.01% as compared to 0.01% (chi square: 60.2; P values less than 0.001), but in this case not significantly lower than in Expt. I. Expts. IIa and IIb do not differ significantly in the percentage of eggs starting development, and when lumped and compared with Expt. I they show a significantly lower starting frequency (chi square 9.3; P = 0.002).

The same female parents were used in Expts. IIa and IIb, and as explained above the females used in the controls and all three experiments came from the same culture bottles. Thus two possible reasons might be given for the percentage of eggs starting development being lower when the eggs were removed to 25°C, as compared to being left at 30°C. Either the higher temperature *does* act on the eggs *after* oviposition, and cause a higher proportion to begin development, or else the fact that the eggs produced in Expts. IIa and IIb came from somewhat older females than those in Expt. I might have had some effect. As noted above, there is no obvious effect of age on rates of parthenogenesis for females kept at 25°C; however, this finding might not hold at 30°C.

It will be noted that with regard to the percentage survival among eggs which *had* begun development, both IIa (7.19%) and IIb (13.21%) show a higher rate of survival than I (3.54%). The P values in the tests of the differences are in both cases less than 0.01. The chi square of the difference between IIa and IIb is 4.69, with a P value of 0.03, indicating that in all probability a prompt removal of the eggs from 30°C as in IIb does raise the rate of survival among those eggs starting development. The survival rates for IIa and IIb do not differ significantly from that of the controls.

As for the percentage of eggs forming viable larvae, it will be noted from table 9 that in both IIa and IIb the net fertility of the virgins, as indicated by production of viable larvae is greater than that of the control virgins, in both cases comparisons of percentage of eggs forming viable larvae with the control figure of 0.08% give chi square values leading to P values of less than 0.01. A comparison of the percentage of viable larvae formed in IIa (0.14%) with that in IIb (0.26%) gives a chi square value of 4.73, leading to a P value of 0.03, and indicating that apparently the early removal of eggs from high temperature as in IIb does result in a significantly greater yield of viable larvae than when they are only removed every twenty-four hours as in IIa.

Thus, to summarize: Maintenance of the virgin females at high tempera-

tures during the time the unfertilized eggs are developed leads to a significantly higher rate of initiation of development followed by a significantly higher mortality rate if the eggs are left at the higher temperature. Prompt removal of the eggs to a moderate temperature has little effect on the higher rate of initiation of development, but results in a significant reduction in the mortality of developing eggs, with the result that the net effect of keeping the virgin females at high temperatures, but removing their eggs to lower temperatures is an approximately three-fold increase in rate of production of viable larvae.

4. *Effect of virginity on parthenogenesis*

A. *Conspecific matings with sterile XO diploid males.* The unisexual strain of *D. parthenogenetica* regularly produced about 1.5% of sterile XO diploid males. In order to test the possible effect of such males on the rate of partheno-

TABLE 10

Tests of parthenogenesis in D. parthenogenetica females mated to sterile XO males. The five females used in this test were kept with two males each throughout the experiment, and only after copulation had been actually observed were egg counts made. Both males and females belonged to generation 9 of the unisexual strain. The control is based on the rate of 69 virgin females of generation 9. (See table 6.)

Female No.	Total eggs	Dead embryos	Larvae	% Total eggs starting development	% Total eggs forming viable larvae
I	925	14	5	2.05	0.54
II	702	10	0	1.42	0.00
III	464	5	1	1.29	0.22
IV	860	7	1	0.93	0.12
V	720	29	2	4.31	0.28
Total	3,671	65	9	2.02	0.25
Control	17,740	327	66	2.22	0.37

genesis, five diploid virgins from generation nine of the unisexual strain were set with two XO males each, in vials, and checked frequently until all five females had been seen in copula. After copulation was observed each female was kept with her mates and changed to fresh food daily; the rate of parthenogenesis being determined over a period of about ten days. The results for the five females are shown in table 10. Although there is a good deal of variability between experimental females, it seems clear that copulation with sterile males has little or no effect on the rate of parthenogenesis.

B. *Conspecific matings with fertile males.* In searching for a possible adaptive value for parthenogenesis in *D. parthenogenetica* the possibility suggested itself that inseminated wild females might, after they had exhausted their sperm supply, continue to reproduce by parthenogenesis, and might then show a considerably higher rate of parthenogenesis than virgin females. Such behavior would have considerable adaptive value in a rare species since then a single insemination would be of potentially very great reproductive value.

If such behavior existed in nature, then laboratory females which had previously mated but had exhausted their sperm supply might be expected to show a higher rate than their virgin sisters. In order to test this possibility nine homozygous garnet virgin females were mated individually to two fertile wild-type males each. These females and their mates were changed to fresh food daily for a week, and until large numbers of larvae indicated that insemination had occurred. At the end of this time the males were discarded and the females maintained in separate vials with daily changes to fresh food until nineteen days after the removal of the males.

Daily counts of the eggs laid each day were recorded, as well as the total numbers of garnet and wild-type males and females produced by each batch of eggs. As indicated above mature larvae reared in vials tend to pupate in the cotton plug, and the pupae so formed to dry out. In order to eliminate this difficulty, in this experiment, after the eggs were counted the vial containing them was unplugged and placed in a half-pint culture bottle which had a double sheet of Kleenex in the bottom which was dampened with water, but was not soaking wet. The culture bottles were capped with cardboard caps. This rather laborious technique almost completely eliminated larval suicide since the mature larvae either pupated at the lip of the unplugged vials or dropped down and pupated in the damp Kleenex on the bottom of the culture bottle. Of the progeny produced from any batch of eggs those which were wild-type females were clearly biparental. The garnet males could have been either biparental or impaternate, but since only 1.5% of the impaternate progeny of diploid virgins are males (see table 1), very little error was introduced by classifying all males as biparental. The garnet females were clearly impaternate (see below). Thus for any one day, subtraction of the number of biparental individuals from the total number of eggs produced left as a remainder the number of unfertilized eggs. Since a small percentage of the fertilized eggs might not have produced adults, this estimate of the number of unfertilized eggs should be if anything too high, and hence the derived rate of parthenogenesis from such unfertilized eggs would be too low.

Actually the error introduced in this way is probably very small since examination of 1,326 eggs from the nine females prior to the elimination of the males showed that of the 982 which actually hatched, 947, or 96.4% produced viable adults. Of the remaining 344 which failed to hatch, only four were detected with dead embryos in them, and this small number of dead embryos could well have been produced parthenogenetically.

In table 11 the results from the nine females are summarized. In this table the individual counts are grouped according to the percentage of unfertilized eggs on a particular day. Thus in the first line in the body of the table are listed the 2,736 unfertilized eggs produced by the nine females on days when no biparental progeny (i.e., 100% unfertilized eggs) were produced; in the second line are listed the 1,506 unfertilized eggs produced on days when from 70% to 99% of the eggs were unfertilized, etc. It will be noted that only 0.10% of the unfertilized eggs produced adults, a figure which is approxi-

TABLE 11

Rates of parthenogenesis in non-virgin females of D. parthenogenetica. Nine females were mated individually to two males each and kept for a week with their mates and until large numbers of larvae were produced, indicating insemination. Then the males were discarded and the numbers of eggs and numbers and kinds of progeny recorded for each female for each day thereafter. The data from the nine females are grouped into categories on the basis of the percentage of eggs unfertilized on any given day. See text.

Percentage of total eggs unfertilized	Number of unfertilized eggs	Number of impaternate adults	Percentage of unfertilized eggs forming adults
100	2,736	4	0.15
70–99	1,506	0	0.00
50–69	537	1	0.19
1–49	326	0	0.00
Total	5,105	5	0.10
Control virgin garnet females	9,281	11	0.12

mately the same as that in the controls. Thus it may be concluded that in so far as conspecific inseminations are concerned, virgin and non-virgin females have approximately the same rates of parthenogenesis.

C. *Exposure to males of other species.* Although mating with conspecific males apparently does not affect the rate of parthenogenesis the possibility still exists that matings with males of other species might have some effect. In *Mollienisia formosa,* HUBBS and HUBBS (1946) have shown that no males of this species are found in nature and that virgin females are apparently sterile. Females will mate with males of congeneric as well as extra-generic species, and either type of mating results in the production of females which are typi-

TABLE 12

Rates of parthenogenesis in D. parthenogenetica homozygous garnet females exposed to males of various species during the egg-laying period. Day-old females were placed with equal numbers of males of the various species and kept with them on fresh food for 10 days prior to testing and throughout the testing period.

		Species of males			
	Control, virgins	*D. polymorpha*	*D. cardini*	*D. simulans*	*D. melanogaster*
No. females tested	57	43	46	62	73
Females inseminated	—	19/38	11/34	—	—
Total eggs	23,371	10,215	13,893	15,808	10,428
Dead embryos	109	18	26	37	18
Larvae	5	4	2	1	1
% Eggs starting development	0.49	0.22	0.20	0.24	0.18
% Eggs forming larvae	0.01	0.04	0.01	0.01	0.01
% Survival among eggs starting development	4.39	18.19	7.14	2.63	5.26

cal *M. formosa.* Thus the females apparently act as reproductive parasites on the males of other species.

It was not practical to make large-scale tests of males of all species believed to be sympatric with *D. parthenogenetica.* However four species were tested: *D. polymorpha, D. cardini, D. simulans* and *D. melanogaster.* Of these four the first two belong to the same species group as *D. parthenogenetica,* the latter two do not. *D. parthenogenetica* has been taken in Atlixco, Mexico, as have *D. cardini, D. simulans* and *D. melanogaster. D. polymorpha* is not known from Mexico, and the most northern locality from which it is recorded is apparently Guatemala (STALKER 1953).

The results of the tests with alien males are recorded in table 12. In these tests homozygous garnet females of *D. parthenogenetica* were kept in vials with equal numbers of alien males for ten days prior to testing. At the time of original exposure to the males the females were one day old while the males varied from one to four days of age. During the test period of approximately one week the flies were kept in vials changed daily, with three pairs of flies per vial. It is at once clear from table 11 that as compared to the garnet control virgin females, the mated females showed no increase in rate of parthenogenesis. In the case of the males of *D. polymorpha* and *D. cardini* matings were frequently observed prior to the test period, and dissection of the *D. parthenogenetica* females confirmed that copulations had been completed, since in the tests with *D. polymorpha* 19/38, and in the tests with *D. cardini* 11/34 of the females were inseminated. Although the males of both *D. simulans* and *D. melanogaster* courted *D. parthenogenetica,* no attempted copulation was observed, and the females were not examined for presence of sperm.

To summarize: For the four species tested it seems clear that unlike the situation in Mollienisia, exposure of virgin females of *D. parthenogenetica* to males of other species has no important effect on the rate of parthenogenesis.

THE RATE OF PRIMARY NON-DISJUNCTION IN D. PARTHENOGENETICA

In the course of early attempts to obtain XXY diploid females by primary non-disjunction of the X-chromosomes in XX diploid females, crosses between homozygous garnet diploid females and wild-type males produced 12,115 diploid progeny. Of these 6,839 were wild-type females, 5,263 were garnet males and 13 were garnet females. There were no wild-type males. The exceptional individuals produced by primary non-disjunction should have been garnet females and wild-type males. This complete absence of exceptional males, although XO males are viable (see table 1), suggested that the 13 garnet females produced were impaternate rather than primary non-disjunctional exceptions. Each of the 13 garnet females was mated to wild-type males, and none of them produced any exceptional progeny, lending force to the assumption that they were impaternate. If primary non-disjunction occurs in this species, it is obviously very infrequent. In *D. melanogaster* it is well known that only non-crossover X-chromosomes show non-disjunction, and the low rate in *D. parthenogenetica* may well be correlated with what appears

in somatic metaphases to be an unusually long X-chromosome. In larval neuroblasts the length of the X-chromosome is about half of the combined lengths of the two major autosomes.

CONCLUSIONS

The mechanism of parthenogenesis

Considering only *D. parthenogenetica,* for which the most complete data are available, it is clear that two related phenomena are involved in effective parthenogenesis.

First, in the unfertilized egg there must be an initiation of cleavage divisions. Secondly, the haploid chromosome complement of the mature egg must be replaced by a diploid or triploid complement. In the case of eggs from diploid females, this last step involves fusion of the haploid meiotic products.

The assumption that cleavage may occur independently of nuclear fusion is based on two facts. 1. The production of 41% diploid males by virgin triploid females can apparently be explained only by the development of unfused XO 2A mature eggs. 2. The very high mortality (80% or higher) of impaternate embryos from diploid females is very difficult to explain except as the result of the production of haploid embryos, since the same females, when crossed to normal fertile XY males of a closely related strain produce almost no dead embryos, thus indicating that the high embryonic lethality can hardly be the result of a lethal gene or genes carried in the stock, and is rather the result of cleavage of eggs without fusion.

While cleavage may occur without nuclear fusion, the data are not critical for answering the following two questions: 1. Is fusion always followed by subsequent cleavage, that is, is some physiological or cytological peculiarity of the egg necessary for both fusion and cleavage, this condition sometimes promoting cleavage only, at other times promoting both fusion and cleavage? 2. Does fusion in itself promote cleavage? Any attempt to answer these two questions with the data at hand entails arguments so lengthy and involved that it does not seem worthwhile to present them here, as they lead to no unequivocal conclusions.

The evolutionary significance of parthenogenesis in Drosophila

In the animal kingdom as a whole, as SUOMALAINEN (1950) has pointed out, the occurrence of thelytoky, or the development of females from unfertilized eggs, is widespread and sporadic, occurring in single species or races rather than being characteristic of larger groups (cyclical thelytoky is an exception to this generalization). This suggests that many of the known cases of thelytoky are of recent origin, and that its actual successful development from bisexual forms must have occurred many times. It is generally supposed (SUOMALAINEN 1950; VANDEL 1936; WHITING 1945) that thelytoky must have arisen from bisexual forms showing occasional or accidental production of females from unfertilized eggs (tychothelytoky). There is some basis for this supposition as indicated by the findings of several workers that ty-

chothelytokous forms occur in bisexual strains, and that in some cases, as in *Solenobia triquetrella,* the few females that do develop from the unfertilized eggs of bisexual strains may themselves produce eggs nearly all of which develop without fertilization. In Drosophila, the marked increase in the rate of parthenogenesis in *D. parthenogenetica* and *D. polymorpha* under severe selection is a less striking example of the same phenomenon. SEILER (1942) believes that in its inception from tychothelytokus forms, thelytoky involves substitution of automixis for fertilization. He further believes that automixis may then gradually be replaced by apomixis involving only one equational maturation division. However, as both SEILER and SUOMALAINEN point out such an intermediate step is not always necessary, since in *Rhabditis pellio* at least, HERTWIG (1920) has observed the sudden origin of an apomictic parthenogenetic race through mutation or segregation in a laboratory stock.

If a tychothelytokous form is to develop into a thelytokous one, a series of rather rare events must occur. First, the impaternate progeny of the tychothelytokous female or females must themselves be sufficiently fertile as virgins to avoid extinction of the unisexual strain. Secondly, the thelytoky, if imperfect at first, as in Drosophila, must be rapidly improved, presumably by selection. Such improvement would involve not only a higher rate of development of unfertilized eggs, but also a higher frequency of nuclear fusion in order to restore the diploid chromosome complement. That selection may result in a higher rate of development of unfertilized eggs is shown by the data for both *D. parthenogenetica* and *D. polymorpha.* In these species the frequency of unfertilized eggs *starting* development in the unselected bisexual strains was 0.9% and 0.3%, respectively, while in the selected unisexual strains the percentages were raised to 8% and 6%, respectively. That selection may result in a higher percentage of nuclear fusions in those eggs *starting* development is indicated by the increased rate of survival. In *D. parthenogenetica* selection increased the survival among impaternate embryos from 9% to 19%, in *D. polymorpha* the survival was increased from less than 2% to 18%.

Even increased initiation of development, and an increased tendency for the fusion of haploid meiotic products is not enough if a large proportion of the fusions involve three rather than two nuclei, and consequent production of the almost completely sterile triploid virgins. Thus if thelytoky were to become functional in a wild strain, the pattern of fusion would have to become modified to largely eliminate triploid production.

Populations of the strain developing functional thelytoky would have to exist in populations which were isolated ecologically or by distance from the bisexual forms, or else selection for thelytoky would be largely neutralized by crosses with fertile bisexual males. Ultimately, of course, reproductive isolation of a rather complete sort might arise in which thelytokous females might refuse to mate with fertile males, or might become structurally or physiologically unable to use the sperm they received in such matings.

In the case of *D. parthenogenetica* practically nothing is known of the ecology or population structure, except that it is rare in collections. Judging

from the feeding habits of other members of the species group it may be supposed that it, too, is a fungus feeder, and the collection data suggest that it is either rare, or very local in its distribution. Locally distributed small populations, would, if they actually exist, furnish the temporary isolation required if a functional thelytokous strain were to develop in nature. Although the very low rate of parthenogenesis observed in the one wild strain first studied makes the development of a wild thelytokous strain seem highly improbable, it must be remembered that the single wild strain representing the species in the laboratory may not indicate the average rate of parthenogenesis in nature, which might be rather high in some individuals. Also, the evidence that thelytoky has developed in other bisexual forms not once, but many times, indicates that the improbable is not the impossible. Thus we feel, that in the case of *D. parthenogenetica,* and probably many other species of Drosophila, the very low rates of parthenogenesis observed are of some potential value to the species in supplying the first step toward thelytoky. Its eventual development depends on the right combination of ecological and genetical factors at the right time.

Polyploidy and parthenogenesis

The repeated discovery of polyploidy in animals among those forms which show thelytoky and the rarity of its discovery in bisexual forms has in a sense verified the prediction made by MULLER (1925) that the XY chromosome mechanism in many bisexual forms would tend to prevent the establishment of polyploid strains. Although exceptions to this general prediction are known, see for example GOLDSCHMIDT (1953) and DARLINGTON (1953), still it seems clear that MULLER's predictions hold to a large extent. In thelytokous species on the other hand, no problem of XY autosome unbalance exists, and one would expect to find polyploids among such forms. For this reason any species which, like *D. parthenogenetica,* may survive by parthenogenesis, is of particular interest in regard to possible development of polyploidy, especially so in this case since the production of one type of polyploids (triploid females), is a regular feature of the parthenogenetic mechanism.

Although in apomictic thelytokous forms, or in automicts in which there is no production of nuclei with a reduced chromosome number and consequently no subsequent nuclear fusion, the establishment of triploids, tetraploids and higher ploid series should occur readily enough; and has occurred; in the case of automicts in which there is a sufficiently normal meiosis to allow for the production of reduced nuclei, with the need for subsequent nuclear fusion, any irregular development of eggs *without* nuclear fusion (as in the formation of haploids from diploid mothers and diploid males from triploid mothers), would in itself constitute a very effective bar to the establishment of a polyploid strain, either in the laboratory or in nature, even if the polyploids, say tetraploids, were themselves viable and fertile. The reason for this is that if, as is the case in the most fertile unisexual laboratory strain of *D. parthenogenetica,* 80% of the developing eggs show no nuclear fusion, then

a tetraploid virgin would produce about four times as many diploid as tetraploid daughters, and unless the tetraploids were greatly superior in vigor or fertility to the diploids, the tetraploidy should disappear as fast as it was produced. As for triploid females, which are known to be produced regularly by diploid virgins, they clearly show a great reduction in fertility, as would be expected in a form producing reduced nuclei in meiosis. Thus in the case of *D. parthenogenetica,* as long as parthenogenesis exists only in its present very imperfect state, with frequent cleavage of eggs without nuclear fusion, then neither in the laboratory nor in nature could a stable polyploid strain be developed.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the capable and painstaking technical assistance of MISS LOIS SCHUERMANN and MISS ELEANOR SUCHLAND during the course of this investigation.

SUMMARY

1. Parthenogenesis is described in the family Drosophilidae. In a survey of twenty-eight members of the family a low rate of parthenogenesis was discovered in twenty-three species. Of these, only three: *D. parthenogenetica, D. polymorpha* and *D. affinis* produced adult progeny; in the remaining twenty species impaternate individuals died in embryonic or larval stages.

2. In *D. parthenogenetica* diploid virgins produce diploid and polyploid daughters as well as rare XO sterile diploid males. The polyploid daughters have been shown to be triploid by a study of their metaphase chromosomes, wing cell size, and breeding behavior. Triploid virgins in this species produce diploid and triploid females and large numbers (40%) of sterile XO diploid males.

3. Diploid virgins of *D. parthenogenetica* heterozygous for the sex-linked recessive garnet (*g*) produce homozygous and heterozygous diploid females as well as +/+/*g* and +/*g*/*g* triploid females. Apparently no +/+/+ nor *g*/*g*/*g*, triploid females are produced in this way. No garnet mosaics have been found.

4. Diploid females crossed to fertile diploid males produce few if any polyploid progeny or primary X-chromosome exceptional individuals.

5. Of the unfertilized eggs from diploid females which start development, 80% die in the late embryonic or early larval stages.

6. It is concluded that the mechanism of parthenogenesis in diploid females involves two normal meiotic divisions followed by fusion of two of the derived haploid nuclei to form diploid progeny, or fusion of three such nuclei to form triploid progeny. In triploid females it is concluded that similar fusions may produce diploid or triploid females but that the large number of diploid XO sterile males are the result of cleavage *without* prior nuclear fusion. Such cleavage without fusion in the eggs of diploid females would lead to the production of haploid embryos and is presumably responsible for the considerable embryonic and early larval death in such eggs.

7. Evidence derived from the progeny of XXY diploid virgins indicates that

binucleate fusion in unfertilized eggs may involve two *terminal* haploid nuclei (those derived from the same secondary oocyte) or two *central* nuclei (those derived from different secondary oocytes).

8. Although certain types of nuclear fusion in the eggs of triploid virgins should result in the production of tetraploid progeny, no wholly tetraploid individuals have been found, suggesting that they are relatively inviable in *D. parthenogenetica.*

9. The establishment of unisexual strains in *D. parthenogenetica* and subsequent rigorous selection for parthenogenesis over a period of seventeen generations has raised the production of viable progeny from the original rate of 8/10,000 to 151/10,000 unfertilized eggs. Similar selection in *D. polymorpha* has increased the rate from 1/19,000 to 133/19,000.

10. When diploid virgins of *D. parthenogenetica* are kept at 30°C for ten days prior to egg production and the freshly laid eggs returned to 25°C, the rate of production of viable progeny is increased approximately three-fold.

11. In *D. polymorpha* diploid virgin females the rate of parthenogenesis (as measured by the production of viable progeny) increases approximately twenty-fold as the females age. No such age-effect is found in *D. parthenogenetica.*

12. Females of *D. parthenogenetica* mated to sterile XO or fertile XY conspecific males, or mated to fertile males of related species show essentially the same rates of parthenogenesis as do virgin females.

13. It is considered that parthenogenesis as it now exists in Drosophila may be of potential value in the eventual production of effectively parthenogenetic strains, although the probability of such an event seems rather remote.

LITERATURE CITED

BRIDGES, C. B., and E. G. ANDERSON, 1925 Crossing over in the X chromosomes of triploid females of *Drosophila melanogaster.* Genetics **10:** 418–441.

CARSON, HAMPTON L., 1946 The selective elimination of inversion dicentric chromatids during meiosis in the eggs of *Sciara impatiens.* Genetics **31:** 95–113.

DARLINGTON, C. D., 1953 Polyploidy in animals. Nature **171:** 191–194.

DOBZHANSKY, TH., 1929 The influence of the quantity and quality of chromosomal material on the size of the cells of *Drosophila melanogaster.* Roux Arch. **115:** 363–379.

FAHMY, O. G., 1952 The cytology and genetics of *Drosophila subobscura.* VI. Maturation, fertilization and cleavage in normal eggs and in the presence of the cross-over suppressor gene. J. Genet. **50:** 486–506.

FRICK, KENNETH E., 1951 Parthenogenetic reproduction in *Phytomyza plantaginis.* Science **114:** 576.

GOLDSCHMIDT, E., 1953 Multiple sex-chromosome mechanisms and polyploidy in animals. J. Genet. **51:** 434–440.

HERING, MARTIN, 1926 Nachgewiesene parthenogenetische Fortpflanzung bei einer blattminierenden acalyptraten Muscidae (Dipt.). Zool. Anzeig. **68:** 283–287.

HERTWIG, P., 1920 Abweichende Form der Parthenogenese bei einer Mutation von *Rhabditis pellio.* Eine experimentelle cytologische Untersuchung. Arch. f. mikr. Anat. **94:** 303–337.

HUBBS, C. L., and L. C. HUBBS, 1946 Experimental breeding of the Amazon Molly. The Aquarium Journal.

HUETTNER, A. F., 1924 Maturation and fertilization in *Drosophila melanogaster*. J. Morph. **39:** 249–265.

MULLER, H. J., 1925 Why polyploidy is rarer in animals than in plants. Amer. Nat. **59:** 346–353.

PATTERSON, J. T., and G. B. MAINLAND, 1944 The Drosophilidae of Mexico. Univ. of Texas Publ. No. 4445: 9–102.

SEILER, J., 1942 Resultate aus der Kreuzung parthenogenetisches und Zweigeschlechtlicher Schmetterlinge. Arch. Klaus-Stift. Vererb-Forsch. **17:** 513–528.

STALKER, HARRISON D., 1951 Diploid parthenogenesis in the cardini species group of Drosophila. Genetics **36:** 577 (Abstract).

1952 Diploid and triploid parthenogenesis in the *Drosophila cardini* species group. Genetics **37:** 628–629.

1953 Taxonomy and hybridization in the cardini group of Drosophila. Ann. Ent. Soc. Am. (in press).

STURTEVANT, A. H., 1923 The probable occurrence of parthenogenesis in *Octhiphila polystigma* (Diptera). Psyche **30:** 22–27.

SUOMALAINEN, ESKO, 1950 Parthenogenesis in animals. Adv. in Genetics **3:** 193–253.

VANDEL, A., 1936 L'evolution de la parthénogenèse naturelle. C. R. XII Cong. Intern. Zool. Lisbonne.

WHITE, M. J. D., 1945 Animal Cytology and Evolution. The University Press. Cambridge, pp. 1-375.

1946 The evidence against polyploidy in sexually-reproducing animals. Amer. Nat. **80:** 610–618.

WHITING, P. W., 1945 The evolution of male haploidy. Quart. Rev. Biol. **20:** 231–260.

WINKLER, HANS, 1920 Verbreitung und Ursache der Parthenogenesis in Pflanzen und Tierreiche. Jena. Verlag von Gustav Fischer.

COAT COLOR INHERITANCE IN HORSES AND IN OTHER MAMMALS

W. E. CASTLE
University of California, Berkeley

Received April 30, 1953

THE colors of horses have long been a subject of interest to owners and breeders of horses as well as to scientists and much information about them is found in the stud-book records of particular breeds. Attempts to use this information in framing explanations of the observed results of matings within or between different color varieties have been made repeatedly since the rise of genetics at the beginning of this century.

Such explanations assume the existence in the primitive undomesticated horse of certain genes for color production which have persisted unchanged in certain domesticated color varieties and undergone mutation in others to produce the genetic combinations recognizable in present-day horses.

Most of the genetic explanations of horse colors have been based on a study solely of horses, ignoring the much larger and more exact knowledge of color inheritance which has been derived from experimental studies made on other mammals, rodents in particular.

A comparative study utilizing this experimental evidence in discussing the genetics of color in horses was made by WRIGHT (1917) and more recently by ODRIOZOLA (1951). Believing this to be a correct method of procedure, I shall attempt in what follows to present a genetic frame-work for explaining horse colors in harmony with that derived from the experimental study of other mammals.

THE COLOR GENE AND ITS ALBINO MUTATIONS

The colors of mammals in general are derived from the presence in the coat of two different groups of pigments which we may call black-brown and red-yellow respectively. Both are end-products of a process of oxidation of a substance or substances capable of producing color when so oxidized.

The initial step in this process is controlled by a hypothetical color gene (symbol C), which may by mutation become inoperative, completely or partially, resulting in albinism. In a complete albino (symbol c), no pigment whatever is formed, so that the hair is white and skin and eyes are pink. Familiar examples of complete albinism are found in white rabbits, rats and mice. Historically the albinism found in white mice was the first mutation in an animal shown to be inherited as a Mendelian recessive character.

A form of incomplete albinism results from a different mutation in the color gene C to an allele designated c^H which when homozygous produces an albino of the sort seen in the Himalayan rabbit. Its body is white and its eyes pink, but the fur on the extremities (ears, nose, tail and feet) is feebly pigmented.

Even the white body coat is capable of developing pigment if kept at subnormal temperatures. This type of albinism, (c^H) as well as complete albinism (c) occurs in domestic rabbits. It occurs also in guinea-pigs and in mink as the only known type of albino in those species. The Siamese cat represents an albino of the Himalayan type, in which considerable sootiness is general in the whitish coat, although the pigmentation is much heavier at the extremities.

A third type of albino mutation, which like the other two is found in rabbits, occurs in the so-called chinchilla rabbit, its gene being designated c^{ch}. It permits pigment to develop in the entire coat, but in a much reduced amount. Experimental crosses show it to be an allele of C, c^H and c, recessive to C but dominant over c^H and c. A zygote may contain any two alleles of the four, but not more. Thus Cc individuals bred together may produce only fully colored and completely albino young; Cc^H individuals may produce only fully colored and Himalayan albino young; Cc^{ch} parents may produce only full colored and chinchilla young; c^Hc parents produce only Himalayan and complete albinos; $c^{ch}c^H$ parents may produce only chinchilla and Himalayan young; and $c^{ch}c$ parents may produce only chinchilla and complete albino young.

There is in horses no certainly demonstrated albino mutation of the color gene, although white horses of at least two different genotypes are commonly known as albinos. These will be discussed later. ODRIOZOLA alone has suggested that one of these is a true albino mutation.

THE BLACK GENE

The coat of most mammals in the wild state is predominantly black pigmented. In the production of black pigment, which is found in the eyes and in the skin of the extremities as well as in the coat, a hypothetical gene B (black) acts in conjunction with gene C. In the absence of any genes which regulate the distribution of black pigment, it will be found throughout the coat.

As a matter of fact very few wild mammals are uniform black in color. This is because of the action of other genes which restrict the distribution of black to particular areas of the coat. When under such gene action black ceases to be produced in a particular area, it is automatically replaced there by red-yellow, a different end product of the oxidation process which occurs in cooperation with gene C. As a result the coat becomes a mosaic of black and yellow pigments which has concealing value in the struggle for existence, allowing a predator to steal upon its victims unobserved, or the prospective victim to escape the notice of the predator.

THE WILD PATTERN GENE

A concealing coat pattern of this sort is the agouti pattern of rabbits and other rodents, which has its counterpart in wolves and many other groups of mammals. Its production is controlled by a gene A (agouti), acting in conjunction with C and B. We may regard this as a gene for wild-coat pattern.

It limits the production of black pigment (1) to particular parts of the coat, chiefly dorsal and peripheral, and (2) also to particular parts of the individual hairs. The result is a generally gray coat dorsally with individual hairs having

an intensely black tip, a sub-apical band of yellow, and below that a dull black base. The ventral areas of the coat are lighter, often lacking the black hair tips, resulting in a whitish yellow belly color and tail white underneath as in the cotton-tail rabbit. This is a very effective concealing pattern in a natural environment. It results entirely from the action of the dominant gene *A* in cooperation with genes *C* and *B*.

Loss of the wild pattern of the coat may result from a recessive mutation of *A* to *a*, leaving the coat a uniform black in the genotype *aaBC*, which is known as non-agouti black or recessive black.

In rabbits and many other rodents, as well as in dogs, the wild-pattern gene *A* has undergone a mutation (designated a^t) which results in the black-and-tan coat pattern. In this mutant, when homozygous (a^ta^t), the individual hairs of the dorsal areas lack the yellow band and so the animal has a black back, a light belly, with banded hairs along the sides between back and belly. This mutation is dominant to black but recessive to ordinary agouti (*A*).

Is there a wild-coat pattern gene in horses comparable with the *A* gene of rodents and other mammals? There certainly is. For the Prejvalski horse, supposedly sole surviving remnant of the wild ancestral stock from which domestic horses arose, is not uniform black in color but has a distinctly concealing pattern. The general body color is a neutral gray probably of mixed black and yellow pigments, but peripheral areas are of black alone including black mane and tail, black dorsal stripe, and black legs.

This pattern is completely lost in the uniform black variety of domestic horses, which we may assume to be of genotype *aaBC*, the ancestral genotype being *ABC*. A domestic variety which certainly retains the wild gene *A* is the bay. All investigators are in agreement that black (*aaBC*) in horses is recessive to bay (*ABC*) just as black in rodents is recessive to agouti.

ODRIOZOLA thinks that the domestic bay retains the wild-pattern gene, not in its original form, but in an allele less effective in producing the wild pattern, just as in rodents a^t is less effective than A in producing the agouti pattern.

This may or may not be so. We have at present no experimental evidence upon it, such as a cross of a Prejvalski horse with a domestic bay might yield.

In rodents and many other mammals, a recessive mutation has occurred in gene *B* to an allele *b*, which when homozygous (*bb*) replaces black pigment with brown throughout the body (eyes, skin and coat). When this happens, the black agouti phenotype becomes brown agouti known as cinnamon (genotype *AbbC*). And the non-agouti or uniform black phenotype (genotype *aaBC*) becomes chocolate in genotype *aabbC*.

A comparable recessive mutation in gene *B* has occurred in horses, giving rise to the chestnut color variety, which was the first demonstrated mendelizing character of horses, as shown by BATESON et al. All investigators are agreed that chestnut is recessive to bay. Their respective genotypes are, in the terminology here adopted, *AbbC*, chestnut; and *ABC*, bay. If we replace *A* in these formulae with its recessive allele *a*, we obtain genotypes *aabbC*, uniform brown, "liver"; and *aaBC*, uniform black, recessive black.

The wild-pattern *A* has been described as one which restricts the distribu-

tion of black pigment in certain portions of the coat or certain parts of individual hairs, where it is replaced by yellow. The result in rodents is the agouti phenotype with its demonstrated concealing character.

The Prejvalski horse also has a concealing coat pattern, as already stated. This pattern, which we assume to be due to the dominant gene *A*, is completely lost in the recessive mutation uniform black, *aaBC*.

A feature of this ancestral pattern is the occurrence, on a prevailingly dull ("dun") colored background, of a dark spinal stripe and bars on legs or withers. This feature, as stated by DARWIN, is often found in ponies. I have recently seen two examples of it among Shetland ponies on a California ranch. Its occurrence among feral horses on the pampas of Argentina is also a well-authenticated historical fact.

THE EXTENSION-RESTRICTION GENE PAIR, *E, e*

In the study of rodents another gene was discovered which further restricts the distribution of black pigment beyond the degree of restriction effected by gene *A*. This gene is designated *e*. It is the supposed recessive allele of a hypothetical dominant gene *E* governing *extension* of black pigment throughout the coat, except in so far as it may be limited by gene *A*.

This new gene when homozygous (*ee*) replaces black or brown pigment with red or yellow in the coat generally, although the eyes, skin of the extremities, and in a minor degree the fur of the extremities may remain black or brown. One investigator has suggested that this be called a *red* mutation, *e* standing for erythros, Greek for red, rather than for restricted distribution of black.

At any rate this mutation results in a generally red or yellow coat, in rabbits and guinea pigs, with the following genetic permutations and phenotypes being clearly distinguishable in rabbits, though in guinea-pigs the agouti and non-agouti yellow phenotypes are less distinct.

ABE, agouti	*AbbE*, cinnamon
ABee, yellow	*Abbee*, yellow (brown eyes)
aaBE, black	*aabbE*, chocolate
aaBee, sooty yellow	*aabbee*, cream (brown eyes)

The question now arises, has a similar recessive red mutation occurred in horses, restricting the distribution of black pigment in the coat beyond the degree of restriction effected by the *A* gene as seen in the Prejvalski horse, and *replacing* black with red in the coat generally?

I would answer this question with yes, pointing to the typical red bay horse as an example. Its general body color is red, not a mixture of black and yellow pigments as in the Prejvalski horse, but it retains the primitive *A* pattern gene as seen in its black mane, tail, and legs; its genotype then may be expressed thus, *ABCee*.

If we adopt this hypothesis, the genotypes of horses corresponding to those of rodents already tabulated will be:

ABE, Prejvalski and ordinary bay
ABee, red-bodied bay
aaBE, black
aaBee, sooty black, " mouse "
AbbE, chestnut
Abbee, sorrel (light mane)
aabbE, liver
aabbee, sorrel (uniform color)

DOMINANT BLACK

In several mammals a dominant mutation of gene *E* to an allele E^D results in a greatly increased production of black pigment and its extended distribution throughout the coat so that it obscures the wild pattern even when heterozygous (EE^D) and completely hides it when homozygous (E^DE^D). This mutation is known as *dominant black*. By contrast *recessive black* lacks the wild-pattern allele *A* and so is of genotype *aaBE*.

This mutation was first observed and has been most carefully studied in rabbits by PUNNETT but has since been shown to occur also in dogs, cats and in the black rat (*Rattus rattus*).

A cross between dominant and recessive black varieties, neither of which shows wild-coat pattern, though one of them carries it hidden by the E^D allele, may result in genotypes:

aaBEE, recessive black
ABEE, agouti
$ABEE^D$, agouti-black, showing traces only of agouti
ABE^DE^D, dominant black, agouti pattern wholly concealed

There is reason to think that in horses, as well as in some other mammals, both dominant and recessive varieties of black occur, and that crosses between them may result occasionally in the unexpected production of a bay colt, an apparent reversal of dominance, but really due to an E^D allele which conceals the *A* pattern more or less completely. Dominant black is probably the commonest type of black found among Shetland ponies, and this may account for the increasing scarcity of the bay color variety among Shetlands, but demonstrative evidence of this interpretation is as yet lacking.

WHITE IN HORSE COLORS

There are horses of many different genotypes which have white or whitish hair on part or all of their bodies.

Among mammals in general the commonest sort of white individual is the albino, completely devoid of pigment in its coat, or nearly so. It arises by a recessive mutation in the color gene *C*, and like all recessives is true-breeding when homozygous.

No true albino mutation has been found among horses, although ODRIOZOLA considers the dilution gene found in Palomino and buckskin horses to belong in this category. Against this view it may be urged that Palomino dilution is not due to a recessive allele of the color gene, but to a dominant modifier of its action, which leaves its latent power unchanged. For when Palomino is mated with Palomino, 25 percent of the colts produced are fully colored chestnut.

Dominant white

The best known type of white horse is one which has a completely white coat but colored eyes. It may be called dominant white, gene *W*. Recent studies of white horses of this sort indicate that gene *W* is probably lethal when homozygous, as is a similar dominant white gene in mice. For no white stallion of this type has failed to produce colored colts when bred to any considerable number of mares either colored or white.

A white stallion, when bred to colored mares, commonly produces 50 percent of colored colts, 50 percent of white colts. When bred to white mares, the expectation is that, if *W* is as suggested lethal when homozygous, the ratio of white to colored colts will be 2:1, instead of 3:1 expected when the parents are heterozygous for a non-lethal dominant gene. This expectation has as yet not been put to an experimental or statistical test.

The physiological action of the *W* gene must be regarded as complete inhibition of color production in the coat, though not in the eye or the skin of certain body regions, which are pigmented.

Dominant dilution

Another type of white horse, not a pure white but ivory white or pale cream in color and with blue eyes, is the so-called cremello, which is homozygous for the dominant dilution gene *D* already described as present in heterozygous combination in Palomino and buckskin horses.

The investigations of WRIEDT (1925, 1928), SALISBURY (1941) and CASTLE and KING (1951) lead to the conclusion that a Palomino horse is heterozygous for a dominant gene for dilution (*D*) and is of genotype *AbbCDd*. Consequently Palominos are not true-breeding. From the mating of Palomino to Palomino there result colts in the ratio of 1dd:2Dd:1DD as regards genotype. The first is chestnut and full-colored, the second Palomino and the third "albino." The "albino" colt has an almost white, ivory or pale cream colored coat, pink skin and blue eyes. It is true-breeding because homozygous, its genotype being *bbDD*. If bred to a chestnut or sorrel mate, it will produce Palomino colts exclusively, all being heterozygotes (*Dd*).

The most desired type of Palomino is one which has a "golden yellow body color with white mane and tail." This suggests that it must have gene *A* in order to reduce the brown pigment in the coat and concentrate it in mane and tail, where it is diluted by gene *D* to a near-white. It suggests also that body color will be rendered clearer, more free from brown pigment, if the genotype is *ee* as in a sorrel rather than *E* as in a chestnut.

The practical recipe for production of the most desired type of Palomino would then be, (1) Secure a Palomino stallion which approaches as nearly as possible the desired type, clear golden yellow body, white mane and tail, genotype *AbbCDdee*. (2) Breed him to sorrel mares with light or flaxen mane and tail, genotype *AbbCee*. Sorrel mates will be preferable to chestnut, because the latter will be *EE* or *Ee* in genotype, whereas the former are all *ee*. All colts will then be *ee*, half of them Palomino, half of them sorrel. Palomino parents

of this type when interbred will produce colts in the ratio 1 sorrel : 2 Palomino : 1 albino, and all the Palominos should be of the desired body color free from sootiness.

If *B* is substituted for *b* in a heterozygous dilute (*Dd*) horse, of the same constitution otherwise as a Palomino, there results a buckskin or "dun" of genotype *ABCDd*. The homozygous (*DD*) genotype produced by interbreeding buckskin horses will be an "albino" with darker pigment in its mane and tail (diluted black) than is found in an "albino" produced by Palominos, which has a diluted brown mane and tail.

The albino produced by interbreeding buckskins may be designated type *B* albino to distinguish it from the albino produced by interbreeding Palominos, called type *A* albino. They differ in genotype only as regards gene *B*, the former carrying the dominant allele, the latter only the recessive.

A dilution gene even more effective in action than gene *D* of Palomino horses has recently been described as occurring in Shetland ponies. This gene is responsible for the production of the silver dapple variety of Shetland ponies and has been designated gene *S*.

Gene *D* modifies brown pigment in Palomino horses more than it modifies yellow-red pigment, which predominates in its body color, modified merely to a "golden-yellow," whereas the color of its mane and tail, in which brown pigment predominates, is modified to such an extent that it is described as "white" by breeders.

Gene *S* like gene *D*, reduces the intensity of black-brown pigment more strongly than red-yellow pigment, but its action is so much more energetic than that of *D* as to reduce black to a shade of "dark cream or light chocolate," while red is reduced in intensity only moderately. So in Shetland ponies of the silver dapple variety mane and tail, in which black pigment would normally predominate, are nearly white, and the body color is a moderately modified red-yellow, which resembles the chestnut body color of horses, for which reason this color variety is also called "dappled chestnut."

Gene *S* of Shetland ponies modifies black to as great an extent as gene *D* modifies brown in Palomino horses. What gene *S* would do to brown pigment in a genuine chestnut pony (*bb*) has not been determined.

In view of the similarity in action of gene *D* in a background of a chestnut or sorrel genotype (*bb*), and that of gene *S* in a background of a black or bay genotype (*B*), it seems possible that the two genes may be alleles. The determination of this point awaits further investigation.

Roan is a coat variation of horses in which white hairs are intermingled with colored ones. The white hairs are present in the coat from birth on and their number remains substantially unchanged. This may be called a non-progressive form of silvering. It is inherited through the agency of a dominant gene *R* for roan. The body color in a roan horse is more often red than black, though both sorts occur, one being called red roan, the other blue roan.

There is reason to think that gene *R*, like *W* for dominant white, is lethal when homozygous. Mr. Frank R. Smith finds (personal communication)

that in mating of roan with roan, a majority of the colts are roan, but the ratio of roan to non-roan is nearer to 2 : 1 than to 3 : 1 and no roan stallion has been found to sire only roan colts, as would be expected of an *RR* individual.

A type of congenital or non-progressive silvering in which the white hairs are localized more or less completely in large patches on the rump is known in the United States as Appaloosa. What its genotype is has not been determined. Non-progressive silvering occurs in cattle and guinea-pigs, comparable with the roan of horses.

Gray is the name given to a progressive type of silvering found in Percherons and in certain other breeds of horses, it results from a dominant gene *G* (gray). The coat of a gray horse, like that of a roan, consists of a mixture of white with colored hairs (mostly black) but the white hairs are not present in the first coat, as they are in roans, but only make their appearance in a later coat and subsequently increase until the coat may become practically all-white at an age of 4–12 years. So this type of silvering is not congenital but progressive, in which two respects it differs from roan.

Unlike *R*, gene *G* is not lethal when homozygous, as gray stallions have been definitely identified which produce gray colts exclusively, whatever the colors of their mates.

Gray horses are usually dappled, but WRIEDT has shown that the character of the dappling (distribution of dark spots in the coat) may be modified by an independent mutation, so it seems probable that dappling is a non-essential and independently determined feature of a gray coat.

Progressive non-congenital silvering occurs in rats as well as in horses but it is recessive in inheritance and so not strictly comparable with the gray of horses.

Many mammals have white-spotted varieties, in which definite areas of the coat are unpigmented. Their occurrence and heritability have been given much attention by students of genetics.

White spotting

In horses minor degrees of white spotting seem to be inherited as recessive characters, such as a star or blaze in the forehead, or one or more white feet. But extensive white spotting, taking the form of large irregular white areas running across the body, is clearly a dominant mutation the gene for which may be called *P* (piebald, or Pinto). Most piebald horses are heterozygous (*Pp*) and when bred to normally colored mates produce 50 percent only of piebald colts.

There is no reason to think that *P* like *W* is lethal when homozygous, but it seems probable that a homozygote would be whiter than a heterozygote, as is certainly the case in dominant white-spotting in rabbits and dogs. Fanciers in such cases prefer the heterozygotes because their pattern is more striking.

SUMMARY

The primitive coat color of most mammals results from the activity of four basic color genes, *A, B, C* and *E*.

1. A black pigment in the eyes, skin and hair results from the interaction of gene *B* (black) with gene *C* (color). The distribution of black pigment in the coat is influenced by a wild-coat-pattern gene *A* (agouti) which in certain regions of the body or in particular parts of individual hairs replaces black pigment with yellow. The result is a concealing or protective coat pattern.

2. The primitive type of coat color dependent upon four dominant genes has become modified in the course of time by recessive mutations in each of them. These form the basis of domestic color varieties many of which would be unable to survive in a wild population because of their lack of concealing value.

3. The first such mutation to be observed in most mammals is albinism resulting from mutation in gene *C* to a recessive allele *c*, which when homozygous (*cc*) results in a completely unpigmented body.

4. The next mutation to be observed is usually a recessive mutation of *A* to *a*, resulting in complete loss of the wild-coat-pattern in a recessive uniform black of genotype *aaBCE*.

5. A recessive mutation of gene *B* to *b* in many mammals results in a qualitative change in the dark pigment from black to brown. There is thus produced a genotype *AbbCE* which in rodents is called cinnamon, in horses chestnut. Combination of this mutation with the foregoing one results in a double recessive genotype (*aabbCE*), a non-agouti uniform brown known as chocolate in rodents, liver in horses.

6. A recessive mutation of gene *E* to *e* effects simultaneously two changes in the coat pigmentation. (a) Extended (*E*) distribution of black or brown pigment becomes restricted (*ee*) distribution and (b) in areas of restricted distribution, red-yellow pigment replaces black or brown. It has been suggested that this secondary effect be emphasized by calling *e* a red mutation (erythros, Greek for red). This mutation when homozygous (*ee*) results in rabbits and guinea-pigs in all-yellow or red body color, black or brown being restricted to the eyes and skin of the extremities. Such *ee* genotypes in horses are represented by the red bay, in which black pigment is restricted principally to mane and tail and legs, and by the sorrel in which brown pigment is similarly restricted.

7. A dominant mutation of *E* to E^D results in a new genotype $ABCE^D$, known as *dominant black*. It is found in rodents, dogs, cats and probably also in horses.

8. Mutations resulting in white hairs or white areas in the coat are found in practically all mammals. In some cases they are recessive, and have little effect on the general coat coloration. In other cases they are dominant and affect the general coat coloration profoundly. In horses the following white hair mutations are known.

(a) Dominant white, coat entirely white, eyes colored. Probably lethal when homozygous, gene *W*.

(b) Dominant dilution, gene *D*, less effective in heterozygotes than in homozygotes. Palomino and buckskin horese are heterozygotes (*Dd*); the corresponding homozygotes (*DD*) are so-called albinos of types A and B respectively.

(c) Dominant dilution, gene *S*, found in silver dapple Shetland ponies reduces the intensity of black pigment as much as gene *D* reduces the intensity of brown pigment. In both cases, black or brown concentrated in mane and tail become almost white.

(d) Roan, gene *R*. A type of congenital, non-progressive silvering, probably lethal when homozygous like gene *W*. A type of congenital, non-progressice silvering in which white hairs are localized on the rump is known in the United States as Appaloosa, genetic basis undetermined as yet.

(e) Gray, gene *G*. A type of dominant non-congenital but progressive silvering usually dappled due to an independent modifier.

(f) Recessive white markings, white feet, blaze, genes undetermined.

(g) Dominant white-spotting, white areas extensive. Gene *P* (Piebald, Pinto). Homozygotes probably whiter than heterozygotes as in similar varieties of white-spotted rabbits and dogs.

LITERATURE CITED

CASTLE, W. E., 1951 Dominant and recessive black in mammals. J. Hered. **42:** 48–59.

1953 Silver, a new mutation of the rat. J. Hered. **44:** (in press).

CASTLE, W. E., and F. L. KING, 1951 New evidence on the genetics of the Palomino horse. J. Hered. **42:** 60–64.

CASTLE, W. E., and FRANK H. SMITH, 1953 Silver Dapple, a unique color variety among Shetland ponies. J. Hered. **44:** 139–145.

ODRIOZOLA, M., 1951 A los colores del caballo. 435 p., illustr. Madrid.

PUNNETT, R. C., 1912 Inheritance of coat colour in rabbits. J. Genet. **2:** 221–238.

1915 Further experiments on inheritance of coat colour in rabbits. J. Genet. **5:** 37–50.

SALISBURY, G. W., 1914 The basic colors and patterns (of the horse). J. Hered. **32:** 235–240; 255–260.

WRIEDT, C., 1925 Vererbungs-untersuchungen beim Pferd. Zeit. ind. Abst. Ver. **38:** 88–101.

1928 Ein neuer Vererbungs-faktor beim Pferd. Hereditas **10:** 274–276.

WRIGHT, S., 1917 Color inheritance in mammals. VII. Horse. J. Hered. **8:** 561–564.

THE MINIATURE COMPLEX IN DROSOPHILA MELANOGASTER

HERMAN M. SLATIS AND DAVID A. WILLERMET
Department of Genetics, McGill University, Montreal, Canada

Received April 30, 1953

ACCORDING to BRIDGES and BREHME (1944), the miniature locus (*m*, I-36.1) was discovered in August, 1910, and the gene has frequently recurred spontaneously and as an induced mutant. Immediately adjacent to *m* is the similar mutant dusky (*dy*), which has been placed at 36.2, although not on the basis of crossover data (STURTEVANT and TAN 1937). Almost no work has been done on these mutants in *Drosophila melanogaster*, and little light has been shed on the relations between them by comparative studies of other species in the genus. STURTEVANT and TAN (1937) also found no crossovers in *D. pseudoobscura*. Reports for *D. virilis* (CHINO 1936), *D. ananassae* (KIKKAWA 1938), and *D. hydei* (SPENCER 1949) show some crossover distance between the two sex-linked loci which are assumed to correspond to *m* and *dy* in these species, but in each case the relative positions have not been tested directly, but have been calculated by mapping with a third locus. Further testing of *D. virilis* (KOMAI and TAKAKU 1949) has resulted in the finding of 8 wild-type flies among 22,334, a number too large to be ascribed to back-mutation (apparently markers were not used to identify crossovers). It may be assumed that an equal number of *m dy* flies occurred but were not recognized, giving a crossover value of about .07%.

The small size of both *m* and *dy* wings is produced in an embryologically similar manner by decreasing the size of the cells (DOBZHANSKY 1929; WADDINGTON 1940). Both also cause a darkening of the wings, and there is a distinct change in the character of the wing epithelium such that moderately large winged flies like dusky may most readily be distinguished from wild type by their failure to reflect light in the same way that the wild type does.

As this paper will show that there is a relationship between *m* and *dy*, these loci will be referred to as the "miniature complex."

MATERIALS AND METHODS

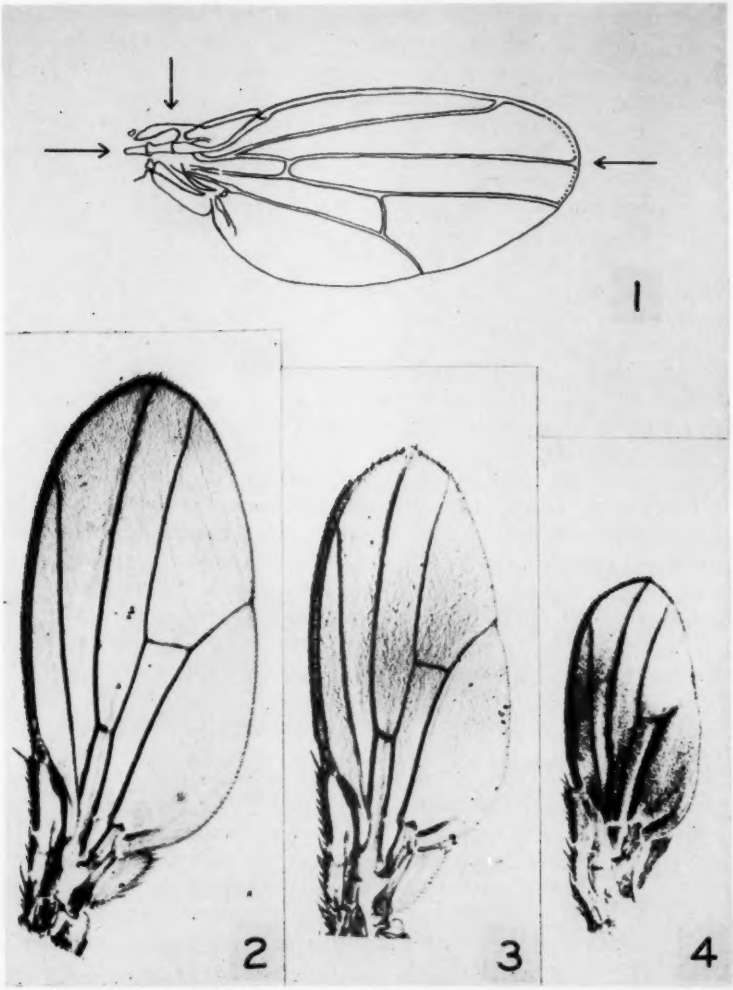
The miniature complex alleles used in this study were *m*, $m^{259\text{-}4}$, m^{D}, and *dy*. The *m* and *dy* alleles are of unknown origin, but may be presumed to be the standard mutants known by these designations, and therefore are of spontaneous origin. The $m^{259\text{-}4}$ allele is an X-ray induced deficiency discovered by DEMEREC. It has been stated to be deficient for *m* but not *dy*. The m^{D} allele was provisionally reported by SLATIS (1949). The following description of this allele may be considered to be definitive.

m^D: miniature-dominant SLATIS, 48k17. From X-ray treatment of *bw; st* male, as one female with a small right wing but a wild-type left wing. No subsequent individuals have shown this mosaicism. Homozygotes about same size as homozygous *m dy* and 5/6 as large as homozygous *m* wing. $m^D/+$ almost precisely between m^D/m^D and +/+. Viability about 20% to 50% in hemizygotes and 5% in homozygotes. Death occurs largely in the egg. Very low fertility in homozygous females. No evidence of a chromosomal aberration from crossover data or salivary gland analysis. Apparently allelic to *m* and *dy*. RK3.

Miniature-dominant may be carried in stocks balanced by *ClB* without any selection. It should prove a useful marker of the center of the X chromosome.

The relative effect of different genotypes was measured by comparing flies from the same bottle whenever possible, since this eliminates the variables which occur between cultures. Except for cytological studies, flies were raised in half-pint bottles at 25°C. The mutants vermilion (*v*, I-33.0) and garnet (*g*, I-44.4) and the *ClB* chromosome were used as markers. Evidence will be presented to support the assumption that these markers have no effect on wing size.

In part of these experiments the wing length was measured from the base of the subcostal vein (leftmost arrow, fig. 1) to the tip of the wing, which is defined as the place where the third longitudinal vein reaches the wing margin (arrow on right, fig. 1). Wing width was measured as the shortest distance across the wing from the point where the fifth longitudinal vein reaches the wing margin. These length and width measurements are not quite perpendicular to each other. At a later time further length measurements were made, but with the proximal point of measurement as the anal end of a thin brown line which occurs within the subcostal vein just proximal to the humeral crossvein (second arrow from the left, fig. 1). These later measurements, though not as good as a measure of length as the earlier ones, are certainly as good an index of it, and are more easily made. In these observations width was not measured. All measurements were made with an ocular micrometer which is divided into two hundred units. Readings were made at about 40×. At this magnification individual micrometer rulings appeared to be just over .01 mm apart and the error of measurement due to misreading the micrometer was quite small. In the later set of observations the right wing was used whenever possible, although the wings of a fly are quite similar in length, without being identical. Probably no more than once in several thousand flies, the wings will differ appreciably (by over .1 mm) and will look unequal on the intact fly, although neither wing appears deformed. Of about 100 flies chosen at random for which both wings were measured, none differed by as much as .03 mm. Those observations in which both length and width were measured were made on cultures begun with several pairs of adults. The bottles in which only length was measured were single pair cultures.



FIGURES 1–4.—The points used in the measurement of wing length and photographs of wings of genotypes $+/+$, $+/m^D$, and m^D/m^D.

RESULTS

Crossover data

The first objective of this project was to determine the crossover distance, if any, between *m* and *dy*. Females of the genotype *v + dy +/+ m + g* or *v m + g/+ + dy +* were crossed with appropriately marked males, and the male offspring were examined. The four possible eye colors, wild, vermilion, garnet and vermilion garnet (which is orange, turning brown with age), are mutually distinguishable. Ocelli color was occasionally used to check for the absence of *v*. Various experiments examined 10,309, 12,318 and 31,091 males. In the first experiment one male had wings smaller than *m* and showed by eye color to be the product of a crossover between *v* and *g*. Subsequent testing found this phenotype to be produced by a condition which is allelic to both *m* and *dy*, and in all manners acts as if a combination of both. At this point it will be sufficient to note that the *m dy* combination is very small winged in hemizygotes and homozygotes. Each of the other two experiments yielded one wild-winged fly which showed by eye color to be the product of a crossover between *v* and *g*. The eye colors of all three of these exceptional flies require that *m* be to the left of *dy*. Some of the above crosses were made in such a manner that the female progeny showed all crossovers between *v*, *m* and *g*. Ninety *m* females which had a crossover which might have produced *m dy* were tested. The results were negative, and as the distance between *m* and *g* is 8.3 units and females bearing the reciprocal crossover were not examined, this is the equivalent of having examined 1084 males. Another test of the linkage of *m* and *dy* was the examination of sons of *m dy/+ +* females. No crossovers were found among 1647 males. Thus, three crossovers were found among the equivalent of 56,449 flies. Because *m* has a lowered viability which is highly variable, and *m dy* is even more capricious, it is difficult to consider these figures as accurate for the estimation of a crossover rate. These data suggest a rate of about one in nineteen thousand, or a crossover value of about .005 units.

The possibility of a crossover between *m* and m^D was investigated by crossing *v* m^D *g/+ m +* and m^D/m females by *m* males. Wild-type crossovers could be seen in progeny of either sex. Of 17,131 progeny examined, one wild-type male was found. This male occurred in a cross in which there were no markers, so that he was either a contaminant, the only back mutation found in these experiments, or evidence that crossing over occurs between *m* and m^D.

No crossovers were found among 5,717 males which might have shown a crossover between m^D and *dy*.

SLATIS (1949) has shown that m^D is not associated with a noticeable change in crossover frequency with *v* and forked (I-56.7).

Salivary gland chromosome analysis

DEMEREC et al. (1942) have found that $m^{259\text{-}4}$ is deficient for the region from 10C3-4 through 10E1-2. This and adjoining regions were examined in

$m^{259\text{-}4}/+$, $m/+$, $dy/+$, $m\ dy/++$, and particularly in $m^D/+$ females. The only aberration found was the deficiency in $m^{259\text{-}4}$.

Wing measurements

Tables 1 to 3 list the measurements made on female progeny from various crosses. Crosses performed in two bottles are listed separately. The markers *v*, *g* and *ClB* have been omitted from the genotypic description of the flies except in special cases (column 1), but either *ClB* or both *v* and *g* were used in each test. The second column lists the number of flies examined in each genotype. The third column gives the average length and its standard error and the fifth column gives the average width and its standard error, expressed in mm. The fourth and sixth columns each gives the probability that the difference in the preceding column is a chance deviation from equality.

The first four crosses in table 1 present the evidence that the *ClB* chromosome probably has no effect on wing size. Neither wing length nor width seems to be affected by the substitution of the *ClB* chromosome for a wild type chromosome. The last three crosses, two of which have been performed twice, show that *dy* might be a complete recessive, but that both *m* and $m^{259\text{-}4}$ distinctly are slightly semidominant, and to about the same extent. The probable semidominance of *m* has been commented on by LUTZ (1913) and CSIK (1934), but neither can be said to have shown $+/m$ to be smaller than $+/+$ by a critical test.

Table 2 is a study of the relationship between *m* and $m^{259\text{-}4}$. The first cross confirms the observation of table 1 that these two mutants are semidominant to about the same degree, although $m^{259\text{-}4}$ may give a slightly smaller and narrower heterozygote than *m*. However, $m^{259\text{-}4}/m$ displays less reduction in wing size than is found in *m* homozygotes. A similar inequality of action is seen in combination with *m dy*, where *m* again produces a stronger effect than $m^{259\text{-}4}$. The next two crosses, the second of which has been done twice, test the allelism of *m* and $m^{259\text{-}4}$ with *dy*. The decrease in wing size from $+\,dy/++$ to $+\,dy/m^{259\text{-}4}+$ is not very much larger than the decrease to be seen from $+\,dy/++$ to $+\,dy/m+$ and may be due to an interaction of *dy* and $m^{259\text{-}4}$ or to chance. At any rate, there is no distinct allelism shown so that we may concur in the opinion that the deficiency does not include the *dy* locus. The last three crosses in table 2 suggest that there is probably no difference in the interaction of *m* and $m^{259\text{-}4}$ with m^D. However, the decrease between $m^D/+$ and m^D/m is twice as large as the decrease which is usually caused by the *m* allele, which can either be interpreted as an interaction between two semidominants acting on the same character, or as a manifestation of allelism.

The first cross in table 3 shows the marked decline in wing size between $m/+$ and m/m. The next cross shows the much smaller decrease for *dy*. The allelism of both *m dy* and m^D with *dy* is shown in the next two crosses. The size of the wings in $m\ dy/+\ dy$ is about what would be expected from the addition of the heterozygous effect of *m* to the *dy* homozygote. This confirms the assumption that *m dy* is merely the coupling phase of these two mutants.

TABLE 1

The effect of heterozygosity for ClB, dy, m and m^{259-4} on wing size.

Genotype	No.	Length ± S.E.	P	Width ± S.E.	P
ClB/+	50	2.3547 ± .0067	.8–.7	.9774 ± .0029	.2–.1
+/+	50	2.3577 ± .0077		.9719 ± .0028	
ClB/m	50	2.2842 ± .0057	.5–.4	.8883 ± .0038	.1–.05
+/m	50	2.2915 ± .0066		.8978 ± .0037	
ClB/dy	50	2.3315 ± .0068	.2–.1	.9846 ± .0034	.9–.8
+/dy	50	2.3196 ± .0061		.9838 ± .0039	
ClB/m^D	50	1.8594 ± .0049	.5–.4	.7093 ± .0028	.9–.8
+/m^D	50	1.8650 ± .0057		.7085 ± .0030	
+/+	50	2.3913 ± .0051	= .4	1.0099 ± .0031	.8–.7
+/dy	50	2.3972 ± .0051		1.0115 ± .0033	
+/+	50	2.3715 ± .0067	.7–.6	.9876 ± .0040	.2–.1
+/dy	50	2.3758 ± .0070		.9958 ± .0038	
+/+	100	2.3998 ± .0026	<.001	.9953 ± .0032	<.001
+/m	100	2.3083 ± .0027		.9454 ± .0031	
+/+	50	2.3737 ± .0073	<.001	1.0134 ± .0042	<.001
+/m^{259-4}	50	2.2725 ± .0056		.9527 ± .0043	
+/+	50	2.3180 ± .0085	<.001	.9824 ± .0095	<.001
+/m^{259-4}	50	2.2210 ± .0084		.9278 ± .0040	

TABLE 2

The relationship between m and m^{259-4}.

Genotype	No.	Length ± S.E.	P	Width ± S.E.	P
+/m	50	2.2746 ± .0071	.1–.05	.9782 ± .0026	= .01
+/m^{259-4}	50	2.2568 ± .0077		.9679 ± .0029	
m/m	50	1.6986 ± .0054	<.001	.6764 ± .0027	<.001
m/m^{259-4}	50	1.8174 ± .0061		.7176 ± .0023	
mdy/m	50	1.5269 ± .0057	<.001	.6058 ± .0041	<.001
mdy/m^{259-4}	50	1.7006 ± .0062		.7093 ± .0037	
dy/+	50	2.3224 ± .0069	<.001	.9988 ± .0045	<.001
dy/m	50	2.2428 ± .0049		.9549 ± .0034	
dy/+	50	2.3509 ± .0088	<.001	.9703 ± .0035	<.001
dy/m^{259-4}	50	2.2135 ± .0084		.9509 ± .0033	
dy/+	50	2.3335 ± .0093	<.001	.9987 ± .0035	<.001
dy/m^{259-4}	50	2.2166 ± .0088		.9477 ± .0035	
m^D/+	50	1.8832 ± .0051	<.001	.7414 ± .0022	<.001
m^D/m	50	1.6646 ± .0082		.6384 ± .0028	
m^D/+	50	1.8879 ± .0052	<.001	.6667 ± .0024	<.001
m^D/m^{259-4}	50	1.6780 ± .0050		.6035 ± .0021	
m^D/m	50	1.6848 ± .0086	= .05	.6322 ± .0045	.9–.8
m^D/m^{259-4}	50	1.6610 ± .0081		.6313 ± .0036	

It also shows that the effect of heterozygous *m* in the presence of a small wing produced by action at another locus, is additive. The lack of interaction between these loci may be interpreted as evidence that the small wing of m/m^D heterozygotes is an effect of allelism rather than of interaction. Miniature-dominant causes much smaller wings than are found in *dy* homozygotes, but the combination dy/m^D is quite a bit smaller than $+/m^D$ and must also be considered as evidence of allelism.

TABLE 3

Allelic relationships of m, dy, m^D and m dy.

Genotype	No.	Length ± S.E.	P	Width ± S.E.	P
+/m	25	2.3024 ± .0107	<.001	.9578 ± .0039	<.001
m/m	25	1.6874 ± .0106		.6532 ± .0040	
+/dy	35	2.3293 ± .0082	<.001	.9877 ± .0042	<.001
dy/dy	35	2.0662 ± .0080		.8755 ± .0029	
dy/dy	50	2.0566 ± .0053	<.001	.8982 ± .0032	<.001
m dy/dy	50	1.9473 ± .0075		.8102 ± .0045	
$+/m^D$	50	1.8424 ± .0045	<.001	.7279 ± .0024	<.001
dy/m^D	50	1.7283 ± .0068		.6713 ± .0031	
+/m	50	2.2024 ± .0058	.5–.4	.9236 ± .0040	.7–.6
dy/m	50	2.1961 ± .0053		.9212 ± .0033	
+/m	50	2.2529 ± .0047	<.001	.9601 ± .0032	<.001
+/m dy	50	2.2123 ± .0057		.9432 ± .0038	
m/m	50	1.6891 ± .0052	<.001	.6551 ± .0023	<.001
m/m dy	50	1.6202 ± .0042		.6294 ± .0027	
m^D/m	50	1.6756 ± .0062	<.001	.6475 ± .0036	<.001
$m^D/m\,dy$	50	1.5289 ± .0073		.5713 ± .0028	
m dy/m	25	1.5901 ± .0097	<.001	.6416 ± .0023	<.001
m dy/m dy	25	1.2893 ± .0085		.5044 ± .0022	
m/m	100	1.6745 ± .0051	.01–.001	.6642 ± .0026	.01–.001
m/m^D	100	1.6949 ± .0044		.6535 ± .0024	
$m^D/m\,dy$	15	1.5545 ± .0077	<.001	.5842 ± .0047	<.001
m^D/m^D	5	1.3960 ± .0130		.5248 ± .0070	
m dy/m dy	15	1.3614 ± .0098	<.001	.5149 ± .0040	<.001
$m\,dy/m^D$	15	1.5725 ± .0063		.5875 ± .0025	

The next cross in table 3 checks on the allelism of *m* and *dy*. In this cross there is a slight drop between *m*/+ and *m* +/+ *dy*, but the difference is not significant. This cross has been repeated in the later group of experiments and comment will be reserved until that time. The next test, which appears to show an enhancement by *dy* of the semidominant effect of *m*, has also been repeated. In this cross there is a greater effect of *m dy* than of *m* alone, and this semidominance of *dy* is also found when it is added to flies homozygous for *m* (giving *m dy*/*m* +). The differences are very small, but are highly significant. The next two crosses, which compare the wing size of *m* and *m dy* when heterozygous with m^D and *m dy*, show a much stronger effect for *m dy*

than for *m*. However, in both cases the difference may be ascribed to allelism at the *dy* locus, and these differences were expected.

Other observations suggested that *m*/*m* and *m*/*m*^{D} would be similar in size, so 100 flies of each type were examined. The differences are not great between the two genotypes, but they are highly significant statistically. It would appear that the allelism between *m* and *m*^{D} is not quite sufficient to bring the heterozygote *m*/*m*^{D} to as small a size as *m*/*m*. The last two crosses in table 3 are concerned with the relation between *m dy* and *m*^{D}. Either homozygote is smaller than the heterozygote, showing that *m dy* and *m*^{D} must depend upon different processes for their phenotypic effect. Each of these mutant types produces wings which are smaller than any previously reported in the miniature complex.

TABLE 4

Average wing length and width and the ratio between them.

Genotype	Length	Width	L/W
+/+	2.37	.99	2.39
+/*dy*	2.35	.99	2.37
+/*m*	2.27	.94	2.41
+/*m*$^{259-4}$	2.25	.95	2.37
m$^{259-4}$ +/+ *dy*	2.22	.95	2.34
m +/+ *dy*	2.22	.94	2.36
+ +/*m dy*	2.21	.94	2.35
dy/*dy*	2.06	.89	2.31
+ *dy*/*m dy*	1.95	.81	2.41
+/*m*^{D}	1.87	.71	2.63
m/*m*$^{259-4}$	1.82	.72	2.53
m^D/*dy*	1.73	.67	2.58
m dy/*m*$^{259-4}$ +	1.70	.71	2.39
m/*m*	1.69	.66	2.56
m^D/*m*	1.68	.64	2.62
m^D/*m*$^{259-4}$	1.67	.62	2.69
m dy/*m* +	1.58	.63	2.51
m^D/*m dy*	1.55	.58	2.67
m^D/*m*^{D}	1.40	.52	2.69
m dy/*m dy*	1.33	.51	2.61

Table 4 summarizes the wing measurements of tables 1 to 3 by giving the unweighted averages for each genotype in descending order of wing length. Wing width and the ratio length/width are also given. The differences in the length/width ratio are not great, but it seems definite that there is a rule (with a single exception) that there is one characteristic width for long wings and a narrower width for short ones.

One object of the second set of wing measurements was to demonstrate the semidominance of *m* and *m dy*. A cross of *v m g*/+ + + females by *v* + *g* males yielded many noncrossover females which could be identified as +/+ or *m*/+ by eye color, and crossover females which could not be identified for their genotype at the *m* locus. These crossover females were measured and progeny tested. Most of the flies which were descended from noncrossovers within the space *v* to *g* were not measured, but ten flies of each genotype from each of

TABLE 5

Wing length of full sisters segregating for dy and +.

Bottle	No.	+/+ ± S.E.	No.	+/*dy* ± S.E.	Diff.	P
1	62	2.198 ± .009	48	2.151 ± .009	.047	<.001
2	58	2.209 ± .009	51	2.176 ± .007	.033	.01–.001
3	10	2.243 ± .008	9	2.195 ± .012	.048	<.001
4	85	2.263 ± .004	83	2.208 ± .006	.055	<.001
5	67	2.195 ± .005	62	2.158 ± .006	.037	<.001
6	51	2.205 ± .006	46	2.140 ± .007	.065	<.001

five bottles gave an average length of 2.22 mm for the wild-type flies and 2.10 mm for the *m* heterozygotes (these and the following measurements were made on a new basis and will be smaller than comparable values in tables 1 through 4). Crossover females were recovered from a total of eighteen bottles. Showing a crossover between *v* and *m*, 23 +/+ females had an average wing length of 2.17 and 22 *m*/+ females had an average length of 2.07. A similar difference is seen if the crossover occurs between *m* and *g*, 61 +/+ females having an average wing length of 2.18 while 57 *m*/+ females had only 2.09 as their average wing length. Comparable data for the *m dy* chromosome showed that 10 noncrossover females of each genotype from each of four bottles had an average of 2.09 among the + +/+ + and 1.96 among the *m dy*/+ +. Also from four bottles, among females containing a crossover between *v* and *m*, four + +/+ + had an average wing length of 2.05 while three *m dy*/+ + had an average of 1.99. Among the crossovers between *dy* and *g*, 15 + +/+ + had a wing length of 2.10 and 13 *m dy*/+ + had an average of 1.97. These measurements clearly show that there is a difference between the wing length of a wild-type fly and one heterozygous for *m* or *m dy*. This difference is almost certainly not due to the action of some other locus. In these experiments the comparison of full sisters precludes most genetic differences and all environmental differences. The consistency of the difference *whether or not* there have been any crossovers in the immediate vicinity of the *m* locus demonstrates that the semidominance is not dependent upon some other locus, including *v* and *g*, which somehow produces the semidominant effect, and which might be postulated to

TABLE 6

Wing length of full sisters of genotypes m+/++ and m+/+dy.

Bottle	No.	*m*+/++ ± S.E.	No.	*m*+/+*dy* ± S.E.	Diff.	P
1	45	2.062 ± .011	49	2.040 ± .012	.022	.2–.1
2	58	2.077 ± .008	60	2.027 ± .008	.050	<.001
3	57	2.057 ± .010	52	2.020 ± .011	.037	.02–.01
4	55	2.088 ± .007	67	2.078 ± .006	.010	.3–.2
5	27	2.091 ± .007	26	2.072 ± .008	.019	.1–.05
6	63	2.071 ± .008	63	2.056 ± .010	.015	.3–.2
7	36	2.097 ± .007	62	2.055 ± .009	.042	<.001
8	46	2.025 ± .012	40	1.990 ± .014	.035	.1–.05
9	72	2.067 ± .006	72	2.033 ± .007	.034	<.001
10	52	2.075 ± .008	62	2.022 ± .008	.053	<.001
11	51	2.058 ± .011	39	2.028 ± .011	.030	.1–.05

be near *m*. The decrease in wing size in *m*/+ flies is not very large. The curve formed by plotting wing lengths in a bottle in which +/+ and *m*/+ are segregating will be bimodal with a large area of overlap. Usually the quarter of the flies with the longest wings will be almost exclusively +/+, and the quarter with the smallest wings will, with a few exceptions, be *m*/+. As wings of aberrant size are more likely to be on the small side than on the large side, it will be simpler to select +/+ individuals from a mixed collection than to select *m*/+. Similar considerations hold for *m dy*, but as *m dy*/+ + is slightly smaller than *m*/+, the amount of overlap will be a little less.

Dusky was tested to see if it is completely recessive, as earlier tests (table 1) had suggested. All non-crossover females were measured in six bottles of the cross *v* + *g*/+ *dy* + by *v* + *g*. The results, which are given in table 5, show a very slight semidominant effect of the *dy* allele, which is consistent with its effects in many crosses. This is also consistent with the findings in *D. virilis* (KOMAI and TAKAKU 1949) where a semidominant effect is found at the two loci assumed to be *m* and *dy* homologues.

TABLE 7

Wing length of full sisters segregating for m and mdy.

Bottle	No.	m+/++ ± S.E.	No.	*mdy*/++ ± S.E.	Diff.	P
1	40	2.060 ± .007	49	2.013 ± .008	.047	<.001
2	29	2.143 ± .008	22	2.085 ± .005	.058	<.001
3	48	2.061 ± .008	49	2.022 ± .009	.039	= .001
4	34	2.107 ± .009	30	2.082 ± .007	.025	.05–.02
5	18	2.121 ± .013	17	2.049 ± .008	.072	<.001
6	40	2.103 ± .010	47	2.041 ± .008	.062	<.001
7	31	2.127 ± .012	24	2.053 ± .009	.074	<.001

Eleven females of the genotype *v* + + *g*/+ + *dy* + were crossed to *v m* + *g* and the offspring females were studied. Adding together all of the noncrossover females, 562 *m* +/+ + had an average wing length of 2.069 while 592 *m* +/+ *dy* had an average wing length of 2.039. The measurements on the noncrossover females in each bottle are presented in table 6. Among the crossovers between *v* and *dy*, 15 *m* +/+ + flies had an average wing length of 2.075 while 13 *m* +/+ *dy* averaged 2.065. Among crossovers between *dy* and *g*, 30 *m* +/+ + averaged 2.100 while 31 *m* +/+ *dy* averaged 2.061. These values are what would be expected as the interaction of the semidominant effects of *m* and *dy*. There are no signs of allelism between these two mutants.

The *m dy* chromosome was tested by crossing *v m* + *g*/+ *m dy* + females by *v* + + *g*. All female offspring from seven crosses were measured. Of the noncrossovers, 240 *m* +/+ + had an average length of 2.097 while 238 *m dy*/+ + had an average length of 2.042. Table 7 presents the results for each bottle. The *m dy*/+ + are consistently smaller than *m* +/+ + and the difference is always statistically significant. The crossover females for which successful progeny tests were accomplished were few in number, but these gave a similar difference between *m* +/+ + and *m dy*/+ +.

A comparison between the mean differences of table 7 and those of table 6

will show a striking difference in order of magnitude. For example, four of the seven differences in table 7 are greater than any of the eleven in table 6. Because of the varying numbers of individuals measured in each group, it is difficult to show the difference between these two bodies of data on statistical grounds. Combined with the observations recorded in table 3, it seems definite that there is a slight difference in expression between the genotype *m dy*/++ and *m* +/+ *dy*, but the confidence level for this conclusion has not been determined.

Viability studies

Croses of m^D/+ females by wild-type males gave 2035 +/+ and 1938 m^D/+ females and 2195 wild and 498 m^D males. Crosses of m^D/+ females by m^D males gave 4563 m^D/+ and 221 m^D/m^D females, and 4704 wild-type and 1157 m^D males. The number of m^D/+ females is very close to that of the +/+, and m^D/+ may be considered to be fully viable. The m^D/m^D homozygotes are much less viable, only .048 as many being found as were expected. The m^D males are more viable than the homozygous females, being found as .2269 and .2460 of the numbers expected. These figures are close to those seen on other occasions, and one may generalize that m^D males appear in between twenty and fifty percent of the numbers expected, while the homozygous females rarely appear in more than five percent of the expected numbers. Observation of eggs, larvae, pupae and adults has shown that the mortality is almost certainly in the eggs.

DISCUSSION

The work of LEWIS (1945) on the Star-asteroid complex began a set of unusual observations which have been continued by LEWIS (1951) on Stubble-stubbloid and the bithorax complex, by GREEN and GREEN (1949) on the lozenge complex, by IVES and NOYES (1951) for singed, and by LEWIS (1952) for white-apricot. Besides these, GREEN (1953) notes that he has similar information for vermilion. LEWIS (1951) found that pseudoallelism is characterized by the genotype *a* +/+ *b* being more deviant from wild type than *a b*/++, where *a* and *b* are " alleles " which cross over. This is true for all thoroughly investigated cases, which are the four complexes worked on by LEWIS, and the lozenge group. A generalized theory of pseudoallelism has been proposed by LEWIS (1951). He assumes that pseudoalleles are really functionally separate genes. The evidence for this is good in his work on the bithorax complex, but is lacking for the other complexes studied. However, since it seems true in one case, it may be accepted as likely in phenotypically similar situations. Essentially, the theory postulates that genes lying in a group take part in successive steps in a synthesis. The gene product is assumed to require the proximity of the next gene for further action so that genes in a particular process must be in the same part of the same chromosome. If a process is blocked by mutation at one point on one chromosome and at another point on the homologue, the process is as effectively blocked as it could be by true alleles. Since crossing over occurs between the apparent alleles, the mutants are said to be pseudo-allelic.

The ease with which measurements may be made on the wings has allowed the collection of more detailed data on the interaction of the miniature complex alleles than has been possible for any of the other pseudoallelic complexes. A number of hypotheses of gene action have been studied which might explain the various wing lengths observed, but none of them are satisfactory. The problems encountered include the following:

1) $m^{259\text{-}4}/+$ is as small or smaller than $m/+$.
2) m/m is much smaller than $m^{259\text{-}4}/m$.
3) $m+/+\,dy$ is almost certainly larger than $m\,dy/++$.

It is usually assumed that a frequently recurring allele such as *m* is the expression of the lack of gene action at a locus (WRIGHT 1929), which has been called an amorph (MULLER 1932). If $m^{259\text{-}4}$ is a deficiency for the locus, then *m* cannot be an amorph (points 1 and 2, above). If we assume that *m* is producing some intermediate amount of the same product the + allele produces (*m* is a hypomorph), then point 1 is explained, but not point 2. If we assume that *m* produces a gene product which is of no value in producing normal wings and that the allele interferes with the normal action of its homologue (*m* is an antimorph), then neither point 1 nor point 2 is satisfied. The simplest explanation would appear to be that $m^{259\text{-}4}$ is not a deficiency for the *m* locus, but is a deficiency which ends adjacent to the *m* locus and is accompanied by a mutation at the locus. This condition is known at the well studied white (*w*) locus, where three deficiences have been interpreted as ending to the left of the locus but are linked with simultaneously induced *w* mutants (GERSH and EPHRUSSI 1946). It is possible that the phenomena responsible for chromosome breakage may also have the ability to mutate adjacent loci. Point 3 is another difficult fact. This observation is directly contradictory to those found in the other cases in *D. melanogaster,* and no reasonable explanation presents itself.

LEWIS's theory of pseudoalleles suggests that such complexes represent separate successive steps in a synthetic process. Presumably, substrate is transformed to an intermediate product by one locus and to a product which may or may not undergo further transformation by another locus. If pseudoalleles are steps leading to an end product, then in most cases at least one phenotype (equivalent to the amorph) should be common to all loci involved in a series. GREEN and GREEN (1949) report that at lozenge, where a large number of alleles are known, similar phenotypes have been observed at each of the three loci. MORGAN et al. (1925) have noted that the *dy* allele in *D. simulans* is more like *m* of *melanogaster* in phenotype than like *dy*. It is possible that the *dy* locus can also have a miniature phenotype, and the striking mutant, m^D may really be at the *dy* locus. Thus far all investigations of pseudoallelic loci have been carried out on multiple allelic loci, but since it appears that at a number of loci (e.g., lozenge) each part of a pseudoallelic locus may retain several phenotypic expressions, it is quite possible that pseudoalleles will be found at frequently mutating loci with only one mutant phenotype.

The phenomenon noted in point 3 suggests that the *m* complex is organized at a different level than the complexes previously studied. The theory of pseudoalleles requires that the site of gene action be the gene itself, rather than some intermediate (enzyme) which is present in more than a single unit in the nucleus or cytoplasm. If the substrate for a gene is common, then the single working unit may be sufficient for the cell, but if the substrate is rare, then there should be a method of channelling it from one gene to the next. For genes which act without intermediates, the chromosome may be an efficient channel. As concentration of product drops rapidly with increase of distance from the gene, the contiguous location of successive steps in a synthesis would be an efficient way of ordering syntheses in which the intermediate products are rare. The *m* complex, representing a step removed from the pattern of close cooperation of other pseudoallelic loci, may be in the process of breaking up a pseudoallelic partnership.

The position of m^D in the *m* complex is uncertain. It is almost certainly allelic both to *m* and *dy*. It may be at the *dy* locus, or at some other locus. A single crossover suggests that it is not at the *m* locus. Because the individual in which m^D first appeared was a mosaic for the character, and such mosaicism has not been seen since, the exact nature of this mutant is in doubt. Being X-ray induced, it may be a small rearrangement (AUERBACH 1951). It is most unusual in being an almost perfect semidominant in phenotype, which is practically unique in *Drosophila*. Its lowered viability is not clearly indicative of a rearrangement. The apparent lack of crossover inhibition argues in favor of it being a point mutation.

SUMMARY

An investigation has been made of the interaction of the mutants *m*, $m^{259\text{-}4}$, m^D and *dy*. Interpretation is based upon measurements of the wing length and width. All of the mutants are semidominant, although only m^D is strongly so. The *m* and $m^{259\text{-}4}$ mutants are allelic, but because the heterozygote $m/m^{259\text{-}4}$ is not as small as *m/m*, it appears possible that $m^{259\text{-}4}$ may be a point mutation associated with a deletion, rather than a deletion which includes the *m* locus. The first 3 crossovers to be reported from *D. melanogaster* place the *dy* locus about .005 unit to the right of *m*. Dusky shows no allelism with *m* and probably none with $m^{259\text{-}4}$. However, the combination *m dy*, which has been recovered from a crossover, almost certainly shows a very slightly greater semidominance than is found in the simple heterozygote between *m* and *dy*, so that *m dy*/+ + is smaller than *m* +/+ *dy*. Confirmatory evidence of some relationship between the *m* and *dy* loci is derived from the fact that the mutant m^D appears to be allelic both to *m* and *dy*. The m^D mutant may be a point mutation. There is evidence that it crosses over with *m*. The *m dy* combination and m^D look alike as hemizygotes or homozygotes (being little more than half as large as wild type and much smaller than any previously known mutant forms at these loci), but are obviously different when heterozygous with wild type or when heterozygous with each other.

The *m* complex does not act in the same manner as the other pseudoallelic loci described from *D. melanogaster*. It is possible that this is a stage in the breakup of what was once a typical pseudoallelic complex. Various theories of the action of the gene have been investigated but no simple explanation of the observations made in this paper can be put forth.

LITERATURE CITED

AUERBACH, C., 1951 Problems in chemical mutagenesis. Cold Spring Harbor Symp. Quant. Biol. **16:** 199–213.

BRIDGES, C. B., and K. S. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Inst. of Wash. Publ. 552: 257 pp.

CHINO, M., 1936 The genetics of *Drosophila virilis*. Japan. J. Genet. **12:** 189–210.

CSIK, L., 1934 Die Zusammenarbeit einiger Gene bei der Determination der Flügelgrösse von *Drosophila melanogaster*. Biol. Zbl. **54:** 614–645.

DEMEREC, M., B. P. KAUFMANN, U. FANO, E. SUTTON and E. R. SANSOME, 1942 The gene. Carnegie Inst. of Wash. Ybk. **41:** 190–199.

DOBZHANSKY, TH., 1929 The influence of the quantity and quality of chromosomal material on the size of the cells in *Drosophila melanogaster*. Arch. Entw. Mech. Org. **115:** 363–379.

GERSH, E. S., and B. EPHRUSSI, 1946 The mechanism of position effect—experiments on the phenotypic expression of position effects in relation to changes in pairing of neighboring chromosome regions. Proc. Nat. Acad. Sci. **32:** 87–94.

GREEN, M. M., 1953 The Beadex locus in *Drosophila melanogaster*: on the nature of the mutants Bx^r and Bx^1. Genetics **38:** 91–105.

GREEN, M. M., and K. C. GREEN, 1949 Crossing-over between alleles at the lozenge locus in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **35:** 586–591.

IVES, P. T., and D. T. NOYES, 1951 A study of pseudo-allelism in two multiple allelic series in *Drosophila melanogaster*. Anat. Rec. **111:** 565.

KIKKAWA, H., 1938 Studies on the genetics and cytology of *Drosophila ananassae*. Genetica **20:** 458–516.

KOMAI, T., and T. TAKAKU, 1949 A study on the closely linked genes, miniature and dusky, in *Drosophila virilis*, with some considerations on the allelism of genes. Cytologia **14:** 87–97.

LEWIS, E. B., 1945 The relation of repeats to position effect in *Drosophila melanogaster*. Genetics **30:** 137–166.

1951 Pseudoallelism and gene evolution. Cold Spring Harbor Symp. Quant. Biol. **16:** 159–174.

1952 The pseudoallelism of white and apricot in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **38:** 953–961.

LUTZ, F. E., 1913 Experiments concerning the sexual difference in the wing length of *Drosophila ampelophila*. J. Exp. Zool. **14:** 267–273.

MORGAN, T. H., C. B. BRIDGES and A. H. STURTEVANT, 1925 The genetics of *Drosophila*. Bibl. Genet. **2:** 1–262.

MULLER, H. J., 1932 Further studies on the nature and causes of gene mutations. Proc. 6th Int. Congr. Genet. **1:** 213–255.

SLATIS, H. M., 1949 m^D: dominant miniature. Dros. Info. Serv. **23:** 63.

SPENCER, W. P., 1949 Gene homologies and the mutants of *Drosophila hydei*. Genetics, Paleontology, and Evolution: 23–44. Princeton Univ. Press.

STURTEVANT, A. H., and C. C. TAN, 1937 The comparative genetics of *Drosophila pseudoobscura* and *D. melanogaster*. J. Genet. **34:** 415–432.

WADDINGTON, C. H. 1940 The genetic control of wing development in *Drosophila*. J. Genet. **41:** 75–140.

WRIGHT, S., 1929 Fisher's theory of dominance. Amer. Nat. **63:** 274–279.

NATURE OF POLYPLOIDY IN SOME 48-CHROMOSOME SPECIES OF THE GENUS SOLANUM, SECTION TUBERARIUM

M. S. SWAMINATHAN[1]
School of Agriculture, University of Cambridge, England

Received May 16, 1953

CHROMOSOME numbers have been reported for 116 species in the section Tuberarium of the genus Solanum. An euploid series with 24, 36, 48, 60 and 72 somatic chromosomes exists. PRAKKEN and SWAMINATHAN (1952) concluded from the available evidence that the basic chromosome number in the genus is 12 and not 6 as suggested by some authors. Species with 48 chromosomes will, on this basis, be tetraploids and tetraploid species are present in the taxonomic series Acaulia Juz., Conicibaccata Bitt., Longipedicellata Buk., and Tuberosa Rydb. Both native and cultivated species with 48 chromosomes occur in the series Tuberosa to which the commercial potato (*Solanum tuberosum*), a tetraploid, belongs. Nineteen tetraploid species are so far known in the section Tuberarium (SWAMINATHAN and HOWARD 1953).

STEBBINS (1947, 1950) has pointed out that it is by no means easy to classify a naturally existing polyploid species into one or the other of the two rigidly contrasting categories usually recognized, namely auto- and allo-polyploids. Such a task is further complicated in potato species since (1) any set of 12 chromosomes is usually capable of pairing with any other set in species hybrids (HOWARD and SWAMINATHAN 1952), and (2) genetic studies are difficult owing to various causes among which the following are some of the more important. First, all potato varieties are heterozygous for many of the characters studied and homozygous plants can only be obtained, if at all, by several years of selfing. Secondly, among the seedlings derived from selfing a variety or from crossing two varieties there is usually a varying percentage of degenerate individuals which may affect genetic segregations for the character being studied. Thirdly, there is the difficulty that among the offspring many may not flower, or, if they do, may have pollen and ovule sterility. Fourthly, segregations are often complex owing to the polyploid constitution of the potato; and fifthly, bud mutations and chimaeras occur frequently. Studies on this problem are nevertheless called for since any information obtained is of considerable interest to potato breeders.

MATERIAL AND METHODS

The following species and varieties possessing 48 chromosomes were used in the study.

[1] Present address: Department of Genetics, University of Wisconsin, Madison, Wisconsin.

Series Acaulia: *Solanum acaule* varieties Bukasov and Recoba.
Series Longipedicellata: *S. longipedicellatum* C.P.C. 28.
Series Tuberosa: *S. andigenum* C.P.C. 1384.
S. tuberosum varieties Duke of York, Gladstone and Cl.B. 72 (the last mentioned is a selection of Mr. J. Clark of Ireland which includes in its parentage the varieties Craigs Defiance, Ballydoon and Katahdin.)

The somatic chromosomes were studied following the use of the 8-hydroxyquinoline pretreatment method of Tjio and Levan (1950). The schedule adopted was somewhat modified (Swaminathan 1950). Meiotic configurations were studied in microsporocytes which had been fixed in acetic alcohol (3 absolute alcohol : 1 acetic acid) to which a few drops of a saturated solution of ferric chloride had been added. Squashing in a drop of acetocarmine gave satisfactory preparations.

Chromosome doubling was induced in *S. acaule, S. longipedicellatum* and *S. tuberosum* by the colchicine-agar seed treatment method (Swaminathan 1950).

Definition of terms

The following terminology as proposed by Stebbins (1947, 1950) is considered to be the most satisfactory.

Autopolyploid: The constituent genomes are completely homologous, the progenitor being a fertile species.

Segmental allopolyploid: Contains two pairs of genomes which possess in common a considerable number of homologous chromosomal segments or even whole chromosomes, but differ from each other in respect to a sufficiently large number of chromosome segments, so that the different genomes produce sterility when present together at the diploid level.

Genomic allopolyploid: Contains two or more sets of very different genomes and the only type of pairing which normally occurs is that between similar chromosomes of the same genome.

Autoallopolyploid: Combines the characteristics of the preceding types. Plants belonging to this group will have the constitution AAAABB, etc.

These terms are used in this paper in the above senses.

RESULTS

a. *Somatic chromosomes.* The somatic chromosomes of *S. tuberosum* (var. Duke of York) and *S. longipedicellatum* (C.P.C. 28) were examined and classified using over-all length, position of centromeres, secondary constrictions and presence of satellites as diagnostic characters. The chromosomes fell into seven different groups (table 1). Each of these species had two chromosomes bearing satellites in their short arms and two chromosomes with secondary constrictions in their long arms (figs. 1 and 2). No conspicuous differences between the idiograms of the two species could be detected.

b. *Meiosis.* The chromosomes are usually associated as bivalents at diaki-

TABLE 1

Somatic chromosomes of S. tuberosum and S. longipedicellatum.

Description	Length in microns	No. of chromosomes in	
		S. tuberosum (Duke of York)	*S. longipedicellatum* (C.P.C. 28)
1. Long chromosomes with sub-median centromere and satellites in the short arm	3.0 to 3.5	2	2
2. Long chromosomes with sub-median centromere and secondary constriction in the long arm	3.0 to 3.5	2	2
3. Long chromosomes with sub-terminal centromere	3.0 to 3.5	8	6
4. Medium length chromosomes with sub-median centromere	2.0 to 3.0	8	8
5. Medium length chromosomes with median centromere	2.0 to 3.0	4	4
6. Short chromosomes with median centromere	1.5 to 2.0	4	0
7. Chromosomes of varying lengths with sub-median centromere	1.0 to 2.5	20	26

nesis and at metaphase I in *S. acaule* and *S. longipedicellatum*. Occasionally, 23_{II} and 2_{I} may be present (figs. 3 and 4). Multivalents occurred in *S. andigenum* and *S. tuberosum* and the mean pairing frequency at M I in these two species was $2.70_{I} + 19.74_{II} + 0.11_{III} + 1.37_{IV}$ (27 cells) and $2.227_{I} + 18.61_{II} + 0.166_{III} + 2.0_{IV}$ (18 cells) respectively. Two M I plates with univalents, bivalents, trivalents and quadrivalents are shown in figures 5 and 6. The chiasma frequency found at M I in the four species examined is given in table 2.

The quadrivalents observed in *S. andigenum* and *S. tuberosum* were of the following types:

Type	Number in *S. andigenum*	Number in *S. tuberosum*
	12	16
	4	5
	21	15

A dicentric bridge and an acentric fragment were twice observed at anaphase I in *S. longipedicellatum* (fig. 7). Metaphase II and subsequent stages were regular in *S. acaule* and *S. longipedicellatum* (fig. 8). In *S. andigenum* and *S. tuberosum*, however, restitution nuclei were formed in 2 to 3% of cells leading to the formation of dyads. Eleven percent of metaphase II plates in *S. andigenum* and 17% in *S. tuberosum* had unbalanced chromosome numbers.

c. *Studies in induced polyploids.* Chromosome doubling was induced by colchicine treatment of seeds in *S. tuberosum* (var. Gladstone), *S. acaule*

FIGURE 1.—Somatic chromosomes of *S. tuberosum*, variety Duke of York (× 1500).
FIGURE 2.—Somatic chromosomes of *S. longipedicellatum*, C.P.C. 28 (× 1200).

(var. Recoba and Bukasov) and *S. longipedicellatum* (C.P.C. 28). Octoploid *S. acaule* and *S. longipedicellatum* plants had thicker leaves with a lower length/breadth index, with more prominent hairs and with larger stomata. They were late to commence flowering and had larger flowers with calyx more hairy, with the pedicels thickened and with the anthers thicker but shorter. These plants were vigorous, fertile and showed a good fruit setting both spontaneously and after crossing with *S. tuberosum* (SWAMINATHAN 1951). In contrast, octoploid *S. tuberosum* plants were dwarf, unhealthy in appearance and never flowered. The chromosome associations at M I of meiosis in octoploids of *S. acaule* and *S. longipedicellatum* were either quadrivalents, bivalents or univalents.

Seeds of F_1 *S. demissum* × *S. tuberosum* (2n = 60) were treated with colchicine to determine the nature of the plants with 120 chromosomes. They were, in general, more vigorous than octoploid *S. tuberosum* plants, formed flowers and set a few seeds (5 seeds per berry).

d. *Studies in hybrids between induced polyploids and S. tuberosum.* 1. Octoploid *S. longipedicellatum* × *S. tuberosum* (Katahdin). This cross succeeded easily whereas no seeds could be obtained following crosses between normal

TABLE 2

Chiasma frequency at M I in tetraploid species.

Species	No. of PMC's examined	No. of configurations with xta					Chiasmata		
		0	1	2	3	4	Total	Per cell	Per bivalent
S. acaule	20	4	173	305	0	0	783	39.15	1.63
S. andigenum	25	73	231	305	16	21	973	36.04	1.50
S. longipedicellatum	15	8	206	150	0	0	506	33.75	1.40
S. tuberosum	18	41	156	202	21	15	663	36.80	1.53

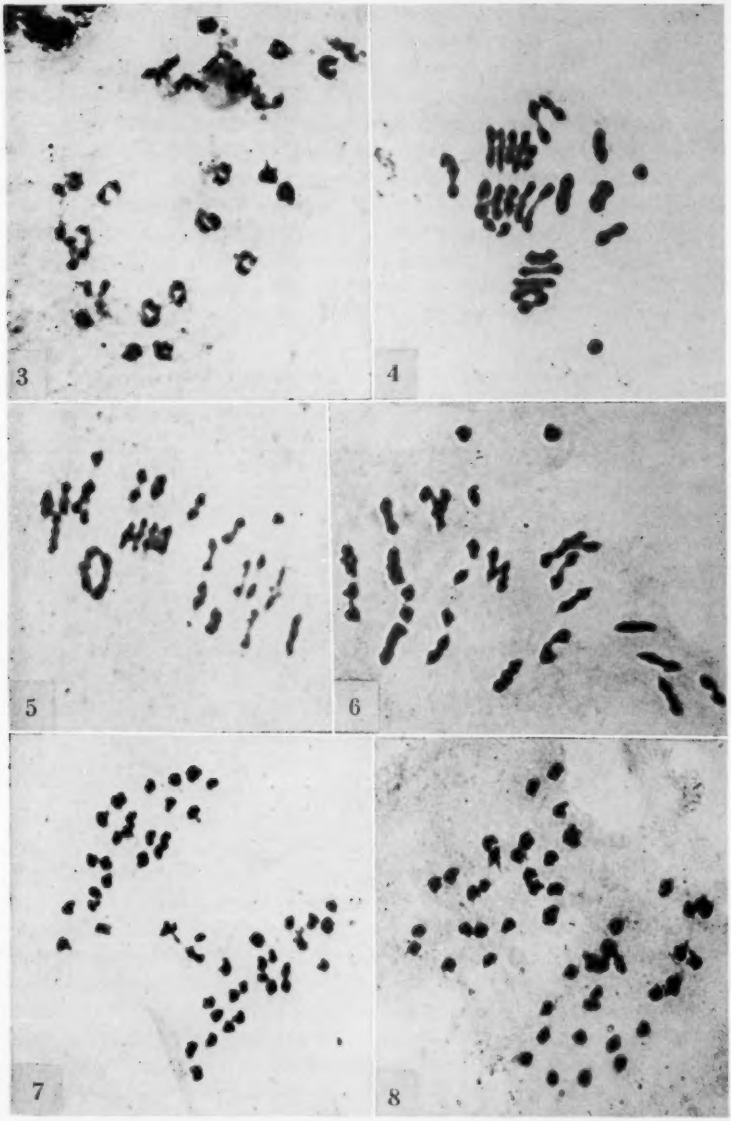


FIGURE 3.—Diakinesis in *S. longipedicellatum*. 24_{II}. FIGURE 4.—First metaphase in *S. longipedicellatum*. $2_I + 23_{II}$. FIGURE 5.—First metaphase in *S. andigenum*. $1_I + 18_{II} + 1_{III} + 2_{IV}$. FIGURE 6.—First metaphase in *S. tuberosum*. $2_I + 19_{II} + 2_{IV}$. FIGURE 7.—First anaphase in *S. longipedicellatum* showing a dicentric bridge and an acentric fragment. FIGURE 8.—Second metaphase in *S. longipedicellatum*. 24 + 24 segregation.

S. longipedicellatum (2n = 48) and *S. tuberosum*. The F_1 hybrids were healthy and flowered but did not produce seeds on selfing. Meiosis was studied in one hybrid plant which had the expected chromosome number 2n = 72. There was at M I a mean frequency per plate of $2.3_{I} + 34.65_{II} + 0.1_{IV}$ (20 cells). The average number of chiasmata per nucleus and per potential bivalent were 49.05 and 1.36 respectively. At M II, each of the 12 plates examined had 36 chromosomes. Seventy-five percent of the pollen was stainable.

The hybrid plants with 2n = 72 would have 48 chromosomes of *S. longipedicellatum* and 24 of *S. tuberosum*. The nearly regular bivalent formation in them may be due to the pairing of the chromosomes of the two species among themselves, since normal *S. longipedicellatum* and haploid *S. tuberosum* (IVANOVSKAJA 1939) form 24 and 12 bivalents respectively.

TABLE 3

Chromosome pairing at M I in (F_1 S. acaule 8x × S. tuberosum) × S. tuberosum, 2n = 60.

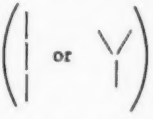
	No. of cells	Configuration				
		I	II	III	IV	V
	1	4	14	6	0	2
	1	3	15	6	1	1
	1	7	13	6	1	1
	1	4	17	6	1	0
	1	14	12	6	1	0
	1	7	17	5	1	0
	1	6	19	4	1	0
	1	8	17	6	0	0
	2	6	18	6	0	0
	1	9	18	5	0	0
	1	7	19	5	0	0
	3	5	20	5	0	0
	1	14	17	4	0	0
	3	8	20	4	0	0
	1	6	21	4	0	0
	1	5	23	3	0	0
	2	7	22	3	0	0
Total	23	159	422	111	6	4
Mean		6.913	18.347	4.826	0.261	0.1765

2. Octoploid *S. acaule* × *S. tuberosum*. Like the octoploid plants of *S. longipedicellatum*, those of *S. acaule* could be easily crossed to *S. tuberosum*, while crosses involving the undoubled plants did not succeed. Thirty-two berries were obtained from the 86 crosses made in 1950 betwen octoploid *S. acaule* and varieties of *S. tuberosum*. Each berry contained, on an average, 31 seeds. The F_1 hybrids, raised in 1951, had broader leaves with many interjected leaflets and a prominent pedicel articulation when compared with *S. acaule* in which the articulation is almost obsolete.

Meiosis was studied in one F_1 plant which had the chromosome number 2n = 72. There was at M I a mean frequency of $1.8_{I} + 34.7_{II} + 0.2_{IV}$ (20 cells). The mean chiasma frequencies per nucleus and per bivalent were 51.15 and

1.42 respectively. Sixty-four percent of the pollen was stainable. The hybrids could be backcrossed to *S. tuberosum* easily and four berries obtained in such crosses had 57, 62, 68 and 91 seeds respectively.

3. (F_1 *S. acaule* × *S. tuberosum*) × *S. tuberosum*. The plants of this cross resembled *S. tuberosum* in leaf shape, pedicel articulation and other morphological characters. One plant was examined cytologically and was found to have 60 somatic chromosomes. There was a mean frequency of $6.91_I + 18.347_{II} + 4.826_{III} + 0.26_{IV} + 0.176_V$ at M I. The detailed data are given in table 3. A striking feature of chromosome association at M I in this plant was the presence of several trivalents (range 3 to 6), which had not been observed in other pentaploid hybrids such as F_1 *S. demissum* × *S. tuberosum* (HOWARD and SWAMINATHAN 1952). There were two pentavalents and six trivalents on one plate (fig. 9). All the trivalents observed were of the chain types



The pentavalents were also simple chains. The mean number of chiasmata per plate was 41.04.

Chromosome distribution was very irregular at M II. Among the 24 cells examined, two had 27 chromosomes; two, 28; four, 29; six, 30; four, 31; two, 32; three, 33; and one, 35. Two plants in which the pollen was examined had 52 and 64% good pollen. These plants could again be easily backcrossed to *S. tuberosum*. Each berry from such a cross contained, on an average, 41 seeds.

The 72-chromosome plants of the cross octoploid *S. acaule* × *S. tuberosum* would have 48 *acaule* and 24 *tuberosum* chromosomes. Their gametes will probably have 24 *acaule* + 12 *tuberosum* chromosomes in view of the fairly regular meiosis in the plants. A pentaploid hybrid resulting from the fusion of such a gamete with a normal *S. tuberosum* gamete will have 24 *acaule* and 36 *tuberosum* chromosomes. The simplest explanation of the occurrence of about 5 trivalents per plate at M I in the pentaploid hybrid would thus be that they are formed following multivalent association of some of the chromosomes of the 3 basic sets of *S. tuberosum* present.

e. *S. acaule* (2n = 48) × *S. simplicifolium* (2n = 24). The triploid F_1 plants from the cross *S. acaule* × *S. simplicifolium* were vigorous and had compound leaves (*S. acaule* has compound leaves and *S. simplicifolium*, simple leaves—fig. 12). There was a mean frequency of $8.85_I + 9.0_{II} + 3.05_{III}$ at M I (20 cells). The plants were both pollen and ovule sterile. Amphidiploid plants were produced by treating F_1 seeds with colchicine (SWAMINATHAN 1950). There were $0.4_I + 35.8_{II}$ per plate (25 cells) at M I in the amphidiploid. At M II, 30 cells had 36 chromosomes, one had 37 and another 35. The subsequent stages were regular. Pollen fertility was about 61%. The plants were self-fertile and set berries spontaneously. The berries contained, on an average, 101 seeds.

The subsequent generations of this amphidiploid grown in 1951 and 1952 consisted of vigorous and fertile plants which were uniform in growth habits.

f. The backcross hybrid (amphidiploid *S. acaule* × *S. simplicifolium*) × *S. simplicifolium*. Crosses were made between amphidiploid *S. acaule* × *S. simplicifolium* and *S. simplicifolium* in 1950. Several of the berries obtained had nothing but shrivelled seeds whereas one berry had 3 good seeds which were sown in 1951. Two seeds germinated and gave rise to healthy plants. One

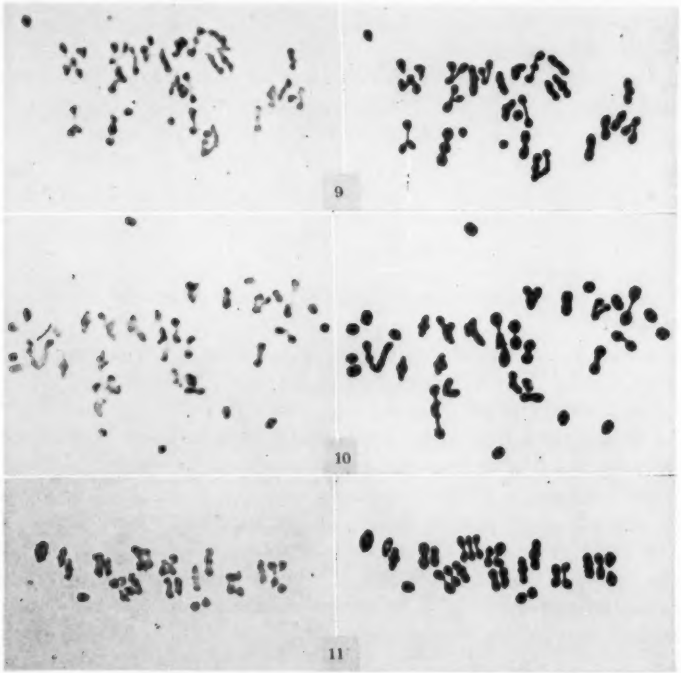


FIGURE 9.—First metaphase in (F_1 *S. acaule* × *S. tuberosum*) × *S. tuberosum*. $4_I + 14_{II} + 6_{III} + 2_V$. FIGURE 10.—First metaphase in (F_1 *S. acaule* × *S. tuberosum*) × *S. tuberosum*. $14_I + 12_{II} + 6_{III} + 1_{IV}$. FIGURE 11.—First metaphase in AD6.151. $3_I + 22_{II}$. (All photomicrographs × 1200.)

(plant no. A.D.6.151) had simple leaves and the other (A.D.6.152) had compound leaves (fig. 12). Chromosome counts in root tips and PMC squashes showed that A.D.6.151 had 2n = 47 and A.D.6.152, 2n = 48.

There was at M I in A.D.6.151 a mean frequency of $4.1_I + 20.1_{II} + 0.9_{III}$ (50 cells). A M I plate of this plant is shown in fig. 11. Fourteen of the 40 M II nuclei studied had 24 chromosomes whereas in the rest, the number ranged from 21 to 27. The plant had 8% good pollen and it produced no seed following either selfing or crosses with the sister plant and *S. tuberosum*. Plant A.D.6.152, on the other hand, was fertile and produced seeds in crosses

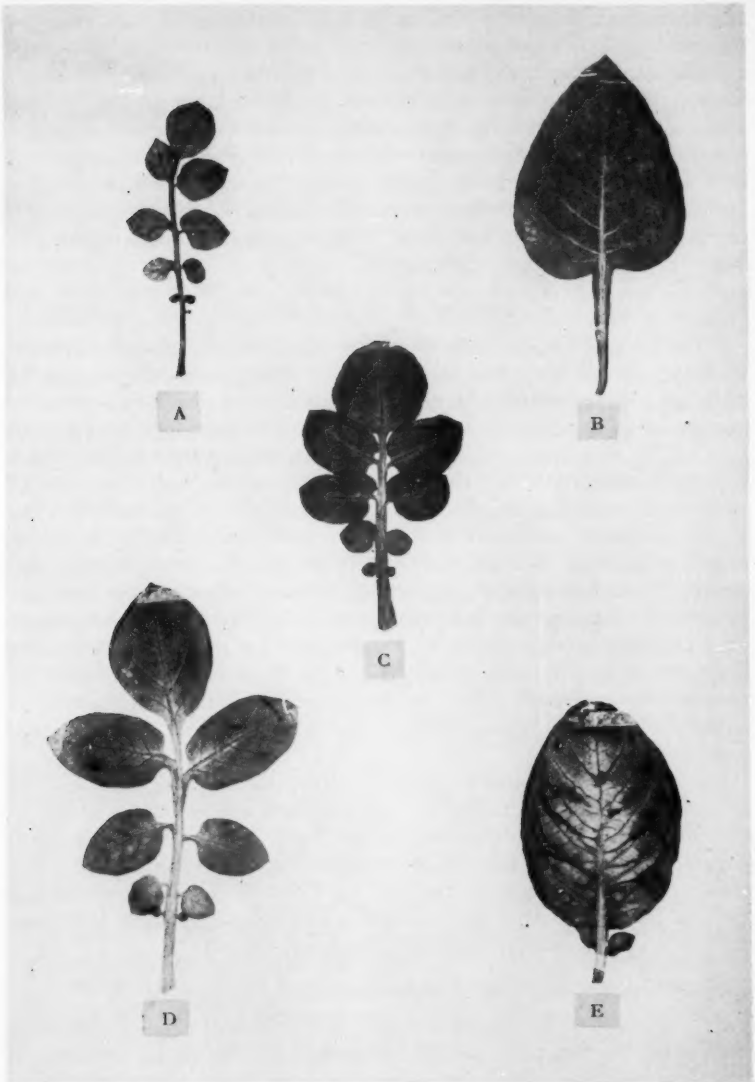


FIGURE 12.—Leaves of A. *S. acaule*; B. *S. simplicifolium*; C. Amphidiploid *S. acaule* × *S. simplicifolium*; D & E. Two plants of the cross (amphidiploid *S. acaule* × *S. simplicifolium*) × *S. simplicifolium*; D. Plant No. AD6.152; E. Plant No. AD6.151.

with *S. tuberosum*. There were $1.2_{I} + 23.08_{II} + 0.08_{III} + 0.1_{IV}$ at M I (50 cells). The chromosome number was regular at M II in 20 of the 24 plates examined. The plant had 61% good pollen.

It was surprising that one plant of this backcross should have 47 chromosomes in view of the rarity of unbalanced gametes in amphidiploid *S. acaule* × *S. simplicifolium*. The cross was repeated in 1951 using the second generation amphidiploid plants as mother parents. Three berries containing a total of 8 seeds were obtained and these seeds were sown in 1952. All of them germinated and the plants had compound leaves and a somatic chromosome number of 48. The plants had 60 to 70% good pollen and in one plant there were $3.6_{I} + 21.4_{II} + 0.4_{IV}$ (5 cells) at M I.

Origin of the simple leaf plant

It was found in studies on the inheritance of leaf type (compound or simple) in diploid species, that those with compound leaves have the genotype *LL* (*L* being a dominant factor for compound leaf) and *S. simplicifolium* has the genotype *ll* (SWAMINATHAN 1952). If *S. acaule*, a tetraploid, has the genotype *LLLL*, its gametes will have *LL* and the triploid hybrid from a cross between it and *S. simplicifolium* will be *LLl*. The amphidiploid will be *LLLLll*. A balanced gamete of the amphidiploid will have *LLl* on the asumption that the 36 bivalents usually formed in it result from pairing within the complements of *S. acaule* and *S. simplicifolium* respectively. The tetraploid plant resulting from backcrossing the amphidiploid to *S. simplicifolium* will have the genotype *LLll* and will have compound leaves. The 47-chromosome backcross plant will have the genotype *Lll* if the gamete of the amphidiploid parent had been deficient for one chromosome with the locus *L*, and should still possess compound leaves since it has been found from studies in amphidiploid *S. macolae* × *S. simplicifolium* that plants simplex for *L*, i.e., *Llll*, have compound leaves.

The formation of a simple leaf plant in the backcross can be explained if *S. acaule* is disomic for *L*. The F_1 hybrid in such a case will be *Ll* and the amphidiploid *LLll*. The balanced gametes of the amphidiploid will have *Ll*, while a deficient gamete with the chromosome carrying *L* absent will have only *l* and such a gamete on being fertilized by *S. simplicifolium* pollen will give rise to a plant with simple leaves. These results thus suggest that with reference to locus *L*, *S. acaule* behaves as a functional diploid.

DISCUSSION

STEBBINS (1950) has described in great detail the occurrence and characteristics of polyploids and has pointed out the difficulties in classifying them. He has recognized four types of polyploids: autopolyploids, segmental allopolyploids, true allopolyploids and autoallopolyploids. These are only modal types and may be connected by several intermediate categories.

Chromosome associations at the first division of meiosis are the usual source of information concerning the type of polyploidy in a given plant. Presence of

multivalents is considered to indicate autopolyploidy and their absence, allopolyploidy. However, MÜNTZING and PRAKKEN (1940) and GILES and RANDOLPH (1951) have proposed for two different reasons that the absence of multivalents should not be treated as evidence of allopolyploidy. The former authors found a genotypically controlled tendency to form only bivalents in an autopolyploid Phleum. The latter authors made a study of the frequency of quadrivalents in a strain of autotetraploid maize at the beginning and end of a ten year period. They found that there were fewer quadrivalents and more bivalents at the end of the period than at the beginning.

The occurrence of tetrasomic ratios of inheritance provides evidence of autotetraploidy. Genetic ratios again may not provide absolute proof of the nature of origin of long established plants since the initially homologous genes lying in the different chromosome sets of a polyploid may mutate in different directions and gradually become so distinct as to be no longer allelic. Such a differentiation may eventually transform a polyploid into a species that has most genes represented only once in the gametes. Also, different loci may segregate differently in segmental allopolyploids so that any conclusions drawn from the study of one or two factors may be misleading.

These limitations will have to be borne in mind while discussing the mode of the origin of a polyploid plant from evidence obtained in present day studies. Such studies, however, show the relationship between the component genomes of a polyploid plant as it exists today, and in a polyploid which is also a crop plant, this knowledge facilitates the formulation of a rational approach to current breeding problems. The available data relating to four Solanum species are summarized below.

S. acaule

This species is highly fertile (pollen and seed) and regularly forms 24 bivalents and no quadrivalents at M I of meiosis. Octoploid *S. acaule* forms quadrivalents and as LAMM (1945) has pointed out, it is hence unlikely that the formation of bivalents in tetraploid *S. acaule* is determined by genotypic control. While no genetic study has been reported in this species, the data presented herein suggest that it behaves as a functional diploid with reference to locus *L*.

The triploid hybrids from crosses between *S. acaule* and diploid species have a high trivalent frequency (3 to 7), which led PROPACH (1937) to suggest that *S. acaule* is really an autotetraploid. However, the fact that amphidiploid *S. acaule* × *S. simplicifolium* forms only bivalents and the hybrid between octoploid *S. acaule* and *S. tuberosum* forms very few quadrivalents, suggest that the two genomes in a gamete of *S. acaule* are well differentiated. No diploid species has so far been found in the taxonomic group Acaulia. JUZEPCZUK (1937) considers Acaulia to be the most recently formed series, since the pedicel articulation is almost obsolete and the corolla lobes are very much fused together. It is probable that *S. acaule* arose as a segmental allotetraploid from diploid species belonging to other taxonomic groups in Solanum.

S. longipedicellatum

This species has two satellited chromosomes, usually forms 24 bivalents and is fertile. One clone was heterozygous for an inversion. Octoploid plants are vigorous, form quadrivalents and are seed fertile. The hybrid between octoploid *S. longipedicellatum* and *S. tuberosum* forms mostly bivalents. Also, as in Acaulia, no diploid species have so far been recorded in the series Longipedicellata. Hence this species seems to be a segmental allotetraploid or a genomic allotetraploid.

S. andigenum

The name *S. tuberosum* was given to the European potato by BAUHIN in 1596 and that name was retained by LINNAEUS. JUZEPCZUK and BUKASOV (1929) grouped the Andean tetraploid varieties into a new species, *S. andigenum*, since these varieties form tubers best under short day conditions and the authors considered that they had had an independent origin from that of *S. tuberosum*. SALAMAN (1937) and HAWKES (1944), however, suggested that the two species were not markedly distinct and that they had had a common origin. VAN DER PLANK (1946) adduced evidence to suggest that the first European potatoes were short day types and he concluded that the new name *S. andigenum* is not justifiable since the chief characteristic of the plants of this species is their short day reaction. HAWKES and DRIVER (1946) agreed with the views of VAN DER PLANK and recently CORRELL (1952) has also listed *S. andigenum* as a synonym of *S. tuberosum*.

Varieties of *S. andigenum* can be easily crossed reciprocally with *S. tuberosum* and the hybrids are fertile. One to two quadrivalents are formed at M I in *S. andigenum* (maximum—4 quadrivalents). The meiotic behavior is hence quite similar to that found in varieties of *S. tuberosum*. Since there could thus be no objection on taxonomic or cytogenetical grounds for *S. andigenum* being considered as a subspecies or variety of *S. tuberosum*, the following discussion as to the nature of polyploidy in *S. tuberosum* would likewise apply to *S. andigenum*.

S. tuberosum

Floral characters. FUKUDA (1927) suggested that *S. tuberosum* arose from a single species since the floral characters in the various varieties have remained constant during the past 300 years. LONGLEY and CLARK (1930) pointed out that there was no need to suppose that more than one type of calyx and corolla were involved in the early ancestry and that it would be possible for a dominant type of flower to be carried unchanged through several generations of inbreeding especially if it was linked with desirable economic characters. All the varieties investigated by LUNDEN (1937) segregated for flower color and this was considered by him to point to the autotetraploid nature of the cultivated potato.

Somatic chromosomes. ELLISON (1935) found that the somatic chromosomes of *S. tuberosum* could not be classified into groups of four by their size.

His results are very misleading since he reported differences between the chromosomes of the varieties Langworthy and Golden Wonder. These should have similar somatic chromosome complements since Golden Wonder has been shown to be a periclinal chimaera with an inner core of Langworthy (CRANE 1936). LAMM (1945) who found 4 chromosomes with satellites in autotetraploid *S. rybinii* suggested that the presence of only two such chromosomes in *S. tuberosum* might be due to amphiplasty (NAVASHIN 1928). It appears that the study of somatic chromosomes will not provide much evidence concerning the nature of polyploidy in Solanum species.

Meiosis. It would appear from the data of authors other than CADMAN (1943)—table 4—that in *S. tuberosum* there occurs a mean trivalent + quadrivalent frequency of between 2 and 3 per plate as compared with about 4 to 6 in artificially produced autotetraploids (SWAMINATHAN 1952) and 0 in *S. acaule* and *S. longipedicellatum*. THOMAS (1946) reported that in *S. tuberosum* only two true quadrivalents per plate occur and he suggested that several

TABLE 4

Frequency of multivalents at M I in S. andigenum and S. tuberosum.

Species and variety	No. of cells with a quadrivalent (+ trivalent) frequency of										Multivalents		Reference
	0	1	2	3	4	5	6	7	8	9	Mean per nucleus	Coefficient of realization	
S. tuberosum													
Flourball	1	0	4	5	13	15	15	11	4	2	5.24	0.436	CADMAN (1943)
Deodara	1	1	3	5	7	3	0	0	0	0	3.25	0.27	LAMM (1945)
36/200	1	8	8	2	1	0	0	0	0	0	1.70	0.14	" "
Cl. B72	1	5	6	3	2	1	0	0	0	0	2.16	0.18	Present study
S. andigenum													
C.P.C.1384	8	8	4	4	3	0	0	0	0	0	1.48	0.12	" "

workers had failed to distinguish between primary and secondary associations. It is difficult to judge whether the quadrivalents observed are formed in a random manner or whether particular chromosomes always form them, since it is not possible to identify the individual chromosomes from morphological characters.

LONGLEY and CLARK (1930), MEURMAN and RANCKEN (1932) and ELLISON (1936) have all suggested that the irregularities of meiosis frequently observed in *S. tuberosum* (like dyad formation, asynapsis, etc.) could be explained if it was assumed that the species had originated as a hybrid between two species whose chromosomes did not pair fully. LAMM (1945) pointed out that similar irregularities occur in synthetic autotetraploids.

Sterility. KOMAROV (1931) suggested that the sterility of many cultivated varieties as contrasted with the fertility of many of the wild species, indicates a hybrid origin of the former. ELLISON (1936) on the other hand, has shown that several cultivated varieties are highly fertile when grown under favorable conditions.

Study of a haploid. IVANOVSKAJA (1939) obtained a single haploid (technically " polyhaploid ") plant of *S. tuberosum* var. Aurora. Usually 12 bivalents were present at M I in this plant but occasionally 11 bivalents and 2 univalents occurred. Bridges and fragments were observed in 7% of the sporocytes at anaphase I, indicating some differentiation of the two genomes in the gametic set of *S. tuberosum*. The haploid produced 3 seeds in 12 berries. IVANOVSKAJA (1939) concluded from these observations that *S. tuberosum* is an allotetraploid. LEWIS (1943) has, however, pointed out that it is to be expected that haploid *S. tuberosum* plants will be self-incompatible like the majority of diploid Solanum species.

Study of species hybrids. The pairing behavior of the chromosomes of the two genomes in the gametic set of *S. tuberosum* can be inferred from hybrids such as the tetraploids obtained from the cross *S. tuberosum* × diploid species (due either to the functioning of unreduced gametes in the diploid parent or the use of autotetraploids of diploids) and from the hexaploid hybrids, octoploid *S. acaule* × *S. tuberosum* and octoploid *S. longipedicellatum* × *S. tuberosum*. All the results agree in showing that the chromosomes of the two genomes may pair to form approximately 12 bivalents. The pentaploid hybrid, (octoploid *S. acaule* × *S. tuberosum*) × *S. tuberosum*, provides some evidence as to the nature of the chromosome association in a triploid complement of *S. tuberosum*. The maximum frequency in a cell of 6 trivalents and 2 pentavalents suggests that 8 chromosomes of each of the 3 sets of 12 can associate together.

Behavior of polyploids. Octoploid plants of *S. tuberosum* are dwarf and weak in contrast to such plants of *S. acaule* and *S. longipedicellatum*, which are large and vigorous. Decaploid (*S. demissum* × *S. tuberosum*, 2n = 120) plants have more vigor than octoploid *S. tuberosum* which may be considered as evidence in support of the general view that species hybrids suffer less from chromosome doubling than true species.

Effect of inbreeding. KRANTZ (1946) found that results from inbreeding commercial potato varieties are more explicable on an autotetraploid than on an allotetraploid basis.

Genetic results. MÜLLER (1930) first pointed out that the potato, owing to its tetraploid nature, does not behave genetically in the same way as a diploid. Prior to that time, all results had been interpreted on a simple, disomic basis. ASSEJEVA and NICOLAEVA (1935) obtained evidence for the occurrence of several duplicate factors controlling plant and tuber color. It appeared probable that some of these duplicate factors were allelomorphic. The occurrence of tetrasomic inheritance in the commercial potato was clearly established by LUNDEN (1937). He found, besides typical tetrasomic ratios of inheritance for certain loci, double reductional segregation, i.e., random chromatid segregation, at the following loci. (a) In reciprocal crosses of the type *Pppp* × *pppp* and *Dddd* × *dddd* (*P* is a factor for purple pigmentation and *D*, a factor needed for the development of plant color), *P* segregated as 1 *P* : 1 *p* while *D* segregated as 13 *D* : 15 *d*. (b) Duplex *RRrr* individuals were found in backcross

progenies of the type *Rrrr* × *rrrr* (*R* is a factor for red color). (c) Nulliplex *dddd* offspring occurred in *DDDd* × *dddd* crosses. CADMAN (1942) found double reductional division at the *Nx* locus (gene *Nx* controls the top necrotic reaction to virus X). Since the publication of LUNDEN'S (1937) paper, segregations for various characters in commercial varieties have been explained on a tetrasomic basis. Simplex genotypes have been assigned in many instances without any other evidence of tetrasomic inheritance and in such cases, it is equally likely that the inheritance is disomic.

To summarize the results of the cytogenetical studies in *S. tuberosum* outlined so far: (a) genetical results suggest that several presumably unlinked loci show tetrasomic inheritance, and (b) cytological results show that first, 12 bivalents are formed in a haploid plant; secondly, a triploid complement may form up to 8 trivalents; and thirdly, a maximum frequency of 5 quadrivalents (9 according to the data of CADMAN 1943) and a mean number of 2 to 3 quadrivalents per metaphase I plate occur in some varieties. The lower quadrivalent frequency at the tetraploid level in comparison with the bivalents in the haploid and trivalents in the triploid may be due to some degree of preferential pairing in the tetraploid caused by a slight differentiation of some of the chromosomes. The extent of differentiation may vary in different varieties probably depending upon the degree of hybridization involved in their evolution. This may lead to significant differences in the multivalent frequencies observed in different varieties. The low chiasma frequency (less than 2 per bivalent) found in Solanum species may also partly lead to the realization of potential quadrivalents in the form of bivalents or trivalents and univalents.

These results would suggest that *S. tuberosum* probably arose as an autotetraploid. However, at this distance from the period of its evolution and with much hybridization work having been done in the breeding of commercial varieties, it would be futile to expect representatives of this species to behave strictly in conformity with the classical definition of autopolyploids. For all practical purposes, we may consider the current commercial varieties as segmental allotetraploids. Whether the term " segmental allotetraploid " can be applied to *S. tuberosum* in the evolutionary sense, as STEBBINS (1950) has proposed, can only be decided after several haploid plants of this species have been obtained and data concerning their fertility are available.

The fact that *S. tuberosum* seems essentially to be an autotetraploid will have some major implications in potato breeding programs. The production of homozygous breeding lines will be a slow process and a good proportion of the inbred plants may be aberrant owing to the possibility for the accumulation of deleterious lethal factors in a vegetatively propagated crop like the potato. It may consequently be difficult to perform several generations of continuous inbreeding. Desirable genes like the ones giving top-necrotic reaction to virus X (COCKERHAM 1943) and resistance to wart (LUNDEN 1950) seem to be present in the varieties possessing them in the simplex state and the breeder will require both much space and patience to get these genes in the triplex or quadruplex conditions. On the other hand, the existence of the possibility to

produce plants quadruplex for a particular gene is a definite advantage in instances where the genes have an additive effect.

SUMMARY

1. *Solanum longipedicellatum* and *S. tuberosum* have two chromosomes with satellites and two with secondary constrictions in their long arms.

2. *S. acaule* and *S. longipedicellatum* invariably form 24 bivalents at metaphase I of meiosis in microsporocytes. Some multivalents ($\pm$ 2) occur in *S. andigenum* and *S. tuberosum*.

3. Octoploid plants of *S. acaule* and *S. longipedicellatum* are vigorous and fertile, while those of *S. tuberosum* are dwarf and fail to flower. Decaploid plants of F_1 *S. demissum* $\times$ *S. tuberosum* with 120 chromosomes are vigorous but weakly fertile. Associations higher than quadrivalents have not been observed in octoploid *S. acaule* and *S. longipedicellatum*.

4. Thirty-six bivalents occur at M I in the hexaploid plants from crosses between octoploid *S. acaule* and *S. longipedicellatum* (pistillate parents) and normal *S. tuberosum* (pollen parent).

5. A maximum frequency of 2 pentavalents + 6 trivalents occurs in the pentaploid hybrid, (F_1 octoploid *S. acaule* $\times$ *S. tuberosum*) $\times$ *S tuberosum*. This suggests that 8 chromosomes of the 3 sets of *S. tuberosum* present in the hybrid may form trivalent associations.

6. The amphidiploid from the cross *S. acaule* $\times$ *S. simplicifolium* is fertile in contrast to the complete sterility of the triploid hybrid. Thirty-six bivalents are present at M I.

7. Two plants from the backcross (amphidiploid *S. acaule* $\times$ *S. simplicifolium*) $\times$ *S. simplicifolium* had the chromosome numbers 2n = 48 and 47 respectively. The 48-chromosome plant had compound leaves like *S. acaule* and the 47-chromosome plant had simple leaves like *S. simplicifolium*. This suggests that *S. acaule* has the factor *L*, needed for the development of the compound leaf, in only 2 of its 4 genomes.

8. The available data suggest that (a) *S. acaule* and *S. longipedicellatum* are segmental allotetraploids, (b) *S. andigenum* and *S. tuberosum* are autotetraploids though the current commercial varieties can be considered as segmental allotetraploids, and (c) both on taxonomic and cytogenetical grounds *S. andigenum* can be treated as a sub-species of *S. tuberosum*.

ACKNOWLEDGMENTS

The author wishes to express his sincere thanks to Dr. G. D. H. Bell, Director, Plant Breeding Institute, Cambridge, for his interest in the problem and for the facilities provided for the investigation; to Dr. H. W. Howard for many discussions; to Prof. R. Prakken and Dr. H. J. Toxopeus, Agricultural University, Wageningen, Holland, for their suggestions and for providing some of the material; and to Dr. D. C. Cooper, Department of Genetics, University of Wisconsin, for his kind help in the preparation of the manuscript.

LITERATURE CITED

ASSEJEVA, T., and N. NICOLAEVA, 1935 Genetic nature of the coloring of potato tubers, sprouts and flowers (In Russian). Works Pot. Res. Inst. Moscow **9:** 107.

CADMAN, C. H., 1942 Autotetraploid inheritance in the potato; some new evidence. J. Genet. **44:** 33–52.

1943 Nature of tetraploidy in cultivated European potatoes. Nature **152:** 103–104.

COCKERHAM, G., 1943 The reaction of potato varieties to viruses X, A, B and C. Ann. Appl. Biol. **30:** 338–344.

CORRELL, D. S., 1952 Section *Tuberarium* of the genus *Solanum* of North America and Central America. U. S. Dept. Agric. Monograph No. 11.

CRANE, M. B, 1936 Note on a periclinal chimaera in the potato. J. Genet. **32:** 73–77.

ELLISON, W., 1935 A study of the chromosome numbers and morphology in certain British varieties of the common cultivated potato (*Solanum tuberosum* L.). Genetica **17:** 1–26.

1936 Meiosis and fertility in certain British varieties of the cultivated potato (*Solanum tuberosum* L.). Genetica **18:** 217–254.

FUKUDA, Y., 1927 Cytological studies on the development of pollen grains in different races of *Solanum tuberosum* with special reference to sterility. Bot. Mag. Tokyo **61:** 459–476.

GILES, A., and L. F. RANDOLPH, 1951 Reduction of quadrivalent frequency in autotetraploid maize during a period of ten years. Amer. J. Bot. **38:** 12–17.

HAWKES, J. G., 1944 Potato collecting expeditions in Mexico and South America. II. Systematic classification of the collections. Imp. Bur. Pl. Breed. Genet. Cambridge, pp. 142.

HAWKES, J. G., and C. M. DRIVER, 1946 Origin of the first European potatoes and their reactions to length of day. Nature **158:** 168.

HOWARD, H. W., and M. S. SWAMINATHAN, 1952 Species differentiation in the section *Tuberarium* of the genus *Solanum* with particular reference to the use of interspecific hybridization in breeding. Euphytica **1:** 20–28.

IVANOVSKAJA, E. V., 1939 A haploid plant of *Solanum tuberosum* L. C. R. (Doklady) Acad. Sci. U.R.S.S. **24:** 517–520.

JUZEPCZUK, S. W., 1937 New species of the genus *Solanum* L. in the group *Tuberarium* Dun. Bull. Acad. Sci. U.S.S.R. Ser. Biol. **2:** 295–331.

JUZEPCZUK, S. W., and S. M. BUKASOV, 1929 A contribution to the question of the origin of the potato. Proc. U.S.S.R. Congr. Genet. Pl. & Animal Breed. **3:** 593–611.

KOMAROV, V. C., 1931 Origin of cultivated plants. State Press of Agricultural & Cooperative Literature, Moscow & Leningrad, pp. 240.

KRANTZ, F. A., 1946 Potato breeding methods. III. A suggested procedure for potato breeding. Minn. Agr. Exp. Sta. Tech. Bul. 173, pp. 24.

LAMM, R., 1945 Cytogenetic studies in *Solanum*, sect. *Tuberarium*. Hereditas **31:** 1–128.

LEWIS, D., 1943 Physiology of incompatibility in plants. III. Autopolyploids. J. Genet. **45:** 171–185.

LONGLEY, A. E., and C. F. CLARK, 1930 Chromosome behaviour and pollen production in the potato. J. Agr. Res. **41:** 867–888.

LUNDEN, A. P., 1937 Arvelighetsundersokelser i potet. Meld. Norg. Landbr. Høisk. **17:** 1–156.

1950 Undersokelser over reaksjon mot kreft (*Synchytrium endobioticum*) hos potet. Meld. Norg. Landbr. Høisk. **137:** 1–48.

MEURMAN, O., and G. RANCKEN, 1932 Untersuchungen über die Chromosomenverhältnisse bei kultivierten Kartoffelsorten (*Solanum tuberosum* L.). Soc. Sci. Fennica Commentationes Biologicae **3:** 1–27.

MÜLLER, K. O., 1930 Ueber die Phytophthora-resistenz der Kartoffel und ihre Vererbung (Zugleich ein Beitrag zur Frage der Polyploidie bei der Kartoffel). Angew. Bot. **12:** 299–324.

MÜNTZING, A., and R. PRAKKEN, 1940 The mode of chromosome pairing in *Phleum* twins with 63 chromosomes and its cytogenetic consequences. Hereditas **26:** 463–501.

NAVASHIN, M., 1928 "Amphiplastie"—eine neue karyologische Erscheinung. Z. Ind. Abst. Ver. Suppl. **2:** 1148–1152.

PRAKKEN, R., and M. S. SWAMINATHAN, 1952 Cytological behaviour of some interspecific hybrids in the genus *Solanum*, sect. *Tuberarium*. Genetica **26:** 77–101.

PROPACH, H., 1937 Cytogenetische Untersuchungen in der Gattung *Solanum*, sect. *Tuberarium*. II. Triploide und tetraploide Artbastarde. Z. Ind. Abst. Ver. **73:** 143–154.

SALAMAN, R. N., 1937 The potato in its early home and its introduction into Europe (Masters Lectures, 1936). J. Roy. Hort. Soc. **62:** 61–266.

STEBBINS, G. L., 1947 Types of polyploids: their classification and significance. Adv. Genetics **1:** 403–429.

1950 Variation and evolution in plants Oxford University Press, pp. 643.

SWAMINATHAN, M. S., 1950 Einige Verfahren für die Verwendung wilder *Solanum*-Arten zu Zuchtzwecken. Züchter **20:** 358–360.

1951 Notes on induced polyploids in the tuber-bearing *Solanum* species and their crossability with *S. tuberosum*. Amer. Potato J. **28:** 472–489.

1952 Species differentiation and the nature of polyploidy in certain species of the genus *Solanum*, sect. *Tuberarium*. Ph.D. dissertation, University of Cambridge, England.

SWAMINATHAN, M. S., and H. W. HOWARD, 1953 The cytology and genetics of the potato (*Solanum tuberosum* L.) and related species. Bibliographica Genetica **16:** 1–192.

THOMAS, P. T., 1946 36th Ann. Report John Innes Hort. Institution, pp. 11.

TJIO, J. H., and A. LEVAN, 1950 The use of oxyquinoline in chromosome analysis. Anales Estacion Experimental de Aula Dei **2:** 21–64.

VAN DER PLANK, J. E., 1946 Origin of the first European potatoes and their reaction to length of day. Nature **157:** 503–505.

HETEROSIS AND THE INTEGRATION OF THE GENOTYPE IN GEOGRAPHIC POPULATIONS OF DROSOPHILA PSEUDOOBSCURA[1]

DANKO BRNCIC

University of Chile, Santiago, Columbia University, New York, and University of Texas, Austin[2]

Received May 26, 1953

THE work on the heterotic state of natural populations of *Drosophila pseudoobscura* has shown that the genotypes of these populations are integrated systems of genes (DOBZHANSKY 1949, 1951; DOBZHANSKY and PAVLOVSKY 1953 and unpublished data). This can be demonstrated most readily when the populations are variable with respect to the gene arrangement in their chromosomes. Inversion heterozygotes which carry two chromosomes derived from any one natural population usually exhibit heterosis, i.e., an adaptive superiority to the corresponding homozygotes. But when the chromosomes are derived from populations of remote localities, the situation becomes considerably more complex. Heterozygotes may or may not show heterosis. In some experiments, in which representatives of geographically different populations are crossed, heterosis is absent at the beginning, but develops gradually in the course of time. This is due to the gene recombination in the offspring of race hybrids producing a great variety of genotypes, some of which possess superior fitness in heterozygous condition. Natural selection acts to perpetuate such superior gene patterns.

The superior fitness, heterosis, of the inversion heterozygotes in any one natural population may be understood on a similar basis. A long-continued process of natural selection has resulted in coadaptation, fitting together, of the gene complexes in the chromosomes which occur in the same local population. The heterosis is a result of an adaptive integration of the population genotype through the action of natural selection.

In the above experiments, the chromosomal inversions have been used as convenient identification tags marking the presence of certain gene complexes in the natural or experimental populations studied. But it seems probable that the fitting together of the gene complexes present in the gene pool of any one population may occur also in the absence of inversions. The phenomenon of adaptive integration of the gene pools of populations may be of quite general occurrence, at least in the higher organisms. The experiments described in the present article represent an attempt to demonstrate the existence of such genotypic integration in the absence of variations in the gene arrangement.

[1] This work was done while the writer held an Inter-American Fellowship provided by the John Simon Guggenheim Memorial Foundation.

[2] Permanent address: Catedra de Biologia, Escuela de Medicina, Universidad de Chile. Borgono 1470. Santiago.

MATERIAL AND METHODS

The initial material consisted of approximately a dozen strains of *Drosophila pseudoobscura* from each of the following localities: Mather, Sierra Nevada, California; Mono Lake, close to the California-Nevada boundary; Lehman Caves National Monument, Nevada; Bryce Canyon National Park, Utah; Ferron, Utah; and Black Canyon of the Gunnison National Monument, Colorado. Each strain descended from a single fertilized female collected in the respective localities by PROFESSOR TH. DOBZHANSKY in summer of 1950. All the strains were known to be homozygous for the Arrowhead gene arrangement in the third chromosomes. Thus the chromosomal variation was completely eliminated from consideration in the experiments to be described. It must however be noted that while in the natural populations of Mono, Lehman, Bryce, Ferron and Gunnison, the Arrowhead homozygotes are the most frequent chromosomal type, they are a minority at Mather (DOBZHANSKY and EPLING 1944; DOBZHANSKY 1948).

The only laboratory strain used in the experiments was one having the dominant marker genes Blade wing (*Bl*) and Scute bristles (*Sc*) in the third chromosome.

All the cultures which served for making counts of the flies hatching in them were kept in incubators at 25°C, except that the oviposition took place at room temperatures. Ten females and ten males were mated per culture; the females were allowed to oviposit for 2 days in each bottle, whereupon they were transferred to fresh cultures. Consequently several bottles with eggs deposited in them by the same group of flies were obtained. Only bottles in which between 60 and 300 flies hatched were considered, and the underpopulated and strongly overpopulated bottles were discarded.

TRANSFER OF THIRD CHROMOSOMES INTO GEOGRAPHICALLY FOREIGN BACKGROUNDS

The *Bl Sc* strain was outcrossed to flies from the Mather and Gunnison stocks. In each case, several *Bl Sc* males were selected from the offspring, and crossed back to the respective geographical strains. Such outcrossings were repeated for 15 generations. As a result, cultures were obtained in which the *Bl Sc* chromosomes were present on the geographic backgrounds of Mather and of Gunnison origin respectively. It should be noted that different strains from Mather and Gunnison were used in the different backcross generations, so that the resulting *Bl Sc* strains carried a variety of modifiers, derived however from a definite geographic population.

Next, several females from various Mather, Mono, Lehman, Bryce, Ferron and Gunnison strains were outcrossed to *Bl Sc* males with the Mather genetic background. A similar series of crosses were made to *Bl Sc* males with the Gunnison genetic background. In the next generation, small groups of *Bl Sc* females and males were selected from each culture and inbred. Wild-type females were taken in the progeny and crossed again to *Bl Sc* males with the Mather background in one series, and with the Gunnison background in the

other. *Bl Sc* females and males were again picked and inbred. This procedure was repeated eight times. As a result, cultures were obtained which carried third chromosomes from Mather, Mono, Lehman, Bryce, Ferron and Gunnison, but which had other chromosomes replaced by those from the Mather strains in one series, and by those from the Gunnison strains in the other series. The possibility of preservation of an admixture of genes from the original stocks is, of course, not excluded, but it is safe to assume that in the main the genotypes of the strains used were as indicated above.

The crosses just described were carried out with great care and patience by MR. BORIS SPASSKY during 1950, 1951 and 1952. The writer profoundly appreciates the generosity of MR. SPASSKY who has permitted the use of the strains prepared by him for the experiments described in the present article.

HETEROSIS PRODUCED BY FOREIGN CHROMOSOMES

Six parallel series of crosses were arranged as follows (fig. 1). In one series, females of pure Mather origin were outcrossed to males with Mather genetic background, but carrying also one *Bl Sc* third chromosome and one third

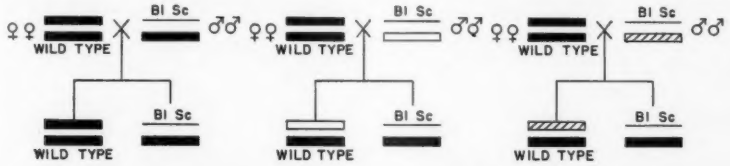


FIGURE 1.—Comparison of the viability of wild-type flies with two third chromosomes of the same geographic origin, with flies heterozygous for two chromosomes of different origins.

chromosome from Mather, or Mono, or Lehman, or Bryce, or Ferron, or Gunnison respectively. The results are shown in the upper part of table 1.

In the second series, females homozygous for Gunnison third chromosomes, but otherwise having Mather genetic background, were crossed to males also with Mather genetic background but carrying *Bl Sc* third chromosomes and third chromosomes from the various populations studied. The results are reported in the middle part of table 1.

The third series was like the second, except that the females carried two third chromosomes of Bryce, instead of Gunnison origin. The data appear in the lower part of table 1.

The fourth, fifth and sixth series of crosses were analogous to the first, second and third respectively, except that all the flies had the genetic background originating from the Gunnison locality. The results are summarized in table 2.

The different crosses of each series are comparable to each other, the only difference between them being the geographic source of the third chromosomes in the male parents. If neither the *Bl Sc* chromosome nor the geographic origin of the other third chromosomes would modify the viability of the flies which

TABLE 1

Results of crosses with the Mather genetic background.

Crosses	Total flies	Percent Wild type	Chi square	P
$\frac{\text{Mather}}{\text{Mather}} \times \frac{Bl\ Sc}{\text{Mather}}$	2146	50.37	....	...
$\frac{\text{Mather}}{\text{Mather}} \times \frac{Bl\ Sc}{\text{Mono}}$	3131	52.31	1.96	0.17
$\frac{\text{Mather}}{\text{Mather}} \times \frac{Bl\ Sc}{\text{Lehman}}$	2139	52.59	2.24	0.14
$\frac{\text{Mather}}{\text{Mather}} \times \frac{Bl\ Sc}{\text{Bryce}}$	2195	53.25	3.54	0.06
$\frac{\text{Mather}}{\text{Mather}} \times \frac{Bl\ Sc}{\text{Ferron}}$	2837	53.08	3.57	0.06
$\frac{\text{Mather}}{\text{Mather}} \times \frac{Bl\ Sc}{\text{Gunnison}}$	2237	49.54	0.29	0.65
$\frac{\text{Gunnison}}{\text{Gunnison}} \times \frac{Bl\ Sc}{\text{Gunnison}}$	4084	56.02	....	....
$\frac{\text{Gunnison}}{\text{Gunnison}} \times \frac{Bl\ Sc}{\text{Mather}}$	3549	55.33	0.28	0.72
$\frac{\text{Gunnison}}{\text{Gunnison}} \times \frac{Bl\ Sc}{\text{Mono}}$	2028	53.40	3.86	0.05
$\frac{\text{Gunnison}}{\text{Gunnison}} \times \frac{Bl\ Sc}{\text{Lehman}}$	2092	60.70	12.50	< 0.001
$\frac{\text{Gunnison}}{\text{Gunnison}} \times \frac{Bl\ Sc}{\text{Bryce}}$	3071	58.35	3.92	0.05
$\frac{\text{Gunnison}}{\text{Gunnison}} \times \frac{Bl\ Sc}{\text{Ferron}}$	2051	57.28	0.86	0.35
$\frac{\text{Bryce}}{\text{Bryce}} \times \frac{Bl\ Sc}{\text{Bryce}}$	2065	51.52	....	....
$\frac{\text{Bryce}}{\text{Bryce}} \times \frac{Bl\ Sc}{\text{Mather}}$	1734	54.90	4.36	0.04
$\frac{\text{Bryce}}{\text{Bryce}} \times \frac{Bl\ Sc}{\text{Gunnison}}$	2708	61.98	51.10	> 0.001

carry them, the progeny of all the crosses would consist of 50 percent *Bl Sc* and 50 percent wild-type flies. This is however not the case, since the *Bl Sc* chromosome tends to reduce the viability of its carriers, while the wild chromosomes increase the viability to different extents.

In the first series of crosses, the replacement of the Mather chromosomes by geographically foreign chromosomes seems to increase the viability in four cases (Mono, Lehman, Bryce, and Ferron), and to decrease it in one case (Gunnison). The differences do not, however, reach the conventional level of

TABLE 2

Results of crosses with the Gunnison genetic background.

Crosses	Total flies	Percent Wild type	Chi square	P
$\frac{\text{Gunnison}}{\text{Gunnison}} \times \frac{Bl\ Sc}{\text{Gunnison}}$	2311	48.72	....	....
$\frac{\text{Gunnison}}{\text{Gunnison}} \times \frac{Bl\ Sc}{\text{Mather}}$	2526	52.57	7.01	0.008
$\frac{\text{Gunnison}}{\text{Gunnison}} \times \frac{Bl\ Sc}{\text{Mono}}$	2363	52.18	5.47	0.02
$\frac{\text{Gunnison}}{\text{Gunnison}} \times \frac{Bl\ Sc}{\text{Lehman}}$	2176	54.87	17.01	< 0.001
$\frac{\text{Gunnison}}{\text{Gunnison}} \times \frac{Bl\ Sc}{\text{Bryce}}$	2280	54.82	17.09	< 0.001
$\frac{\text{Gunnison}}{\text{Gunnison}} \times \frac{Bl\ Sc}{\text{Ferron}}$	2255	53.03	8.41	0.003
$\frac{\text{Mather}}{\text{Mather}} \times \frac{Bl\ Sc}{\text{Mather}}$	2876	48.64	....	....
$\frac{\text{Mather}}{\text{Mather}} \times \frac{Bl\ Sc}{\text{Mono}}$	2132	49.39	0.26	0.70
$\frac{\text{Mather}}{\text{Mather}} \times \frac{Bl\ Sc}{\text{Lehman}}$	2010	54.57	16.57	< 0.001
$\frac{\text{Mather}}{\text{Mather}} \times \frac{Bl\ Sc}{\text{Bryce}}$	2682	51.08	3.35	0.07
$\frac{\text{Mather}}{\text{Mather}} \times \frac{Bl\ Sc}{\text{Ferron}}$	2284	51.40	3.87	0.05
$\frac{\text{Mather}}{\text{Mather}} \times \frac{Bl\ Sc}{\text{Gunnison}}$	2917	56.53	35.50	< 0.001
$\frac{\text{Bryce}}{\text{Bryce}} \times \frac{Bl\ Sc}{\text{Bryce}}$	2480	50.24	....	....
$\frac{\text{Bryce}}{\text{Bryce}} \times \frac{Bl\ Sc}{\text{Mather}}$	2206	53.49	4.95	0.025
$\frac{\text{Bryce}}{\text{Bryce}} \times \frac{Bl\ Sc}{\text{Gunnison}}$	2598	55.42	13.77	< 0.001

statistical significance, although they approach it in the cases of Bryce and Ferron (table 1). In the second series, making the two third chromosomes of different geographic origin gives a significant improvement of the viability in one case (Lehman), an improvement which does not reach the significant level in two cases (Bryce and Ferron), and a nonsignificant deterioration in two cases (Mono and Mather). In the third series, making the two third chromosomes different in geographic origin improves the viability quite significantly in the two cases tried (Mather and Gunnison) (table 1).

The experiments with the Gunnison genetic background, i.e., the fourth to sixth series (table 2), show an improvement of the viability in every one of the twelve crosses in which the two third chromosomes which the flies carried were made different in geographic origin. This improvement is statistically quite significant in ten cases, and close to the borderline of significance in one.

It should be noted that the results show no trace of a tendency towards improvement of the viability when the third chromosomes agree in geographic origin with the remainder of the genotype. Such an effect would not be unexpected, since the abnormalities in the spermatogenesis of *Drosophila pseudoobscura* × *Drosophila persimilis* hybrid males are the greater the more mixed are the chromosomes in their karyotype (DOBZHANSKY 1951). In reality, the flies which have only Mather chromosomes (the top of table 1), or only

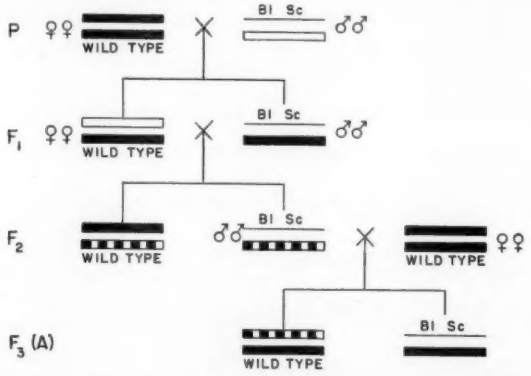


FIGURE 2.—Experiments designed to test the effects of crossing-over between chromosomes of different geographic origin (shown in black and white) on the viability. Flies with entire third chromosomes from different populations (F_1) are compared with flies that carry one chromosome resulting from crossing over with (F_2(A)).

Gunnison chromosomes (the top of table 2), are close to the bottom of the viability scale recorded in these experiments.

THE EFFECTS OF CROSSING OVER BETWEEN CHROMOSOMES OF DIFFERENT GEOGRAPHIC ORIGIN

In the experiments described so far, the chromosomes of various geographic origins were treated as units. Crossing over between chromosomes of different origins was prevented by using male heterozygotes, in which crossing over is absent or very rare. The experiments now to be dealt with involve, on the contrary, the break-up of the gene complexes of various geographic origins.

The type of experiments represented schematically in figures 2 and 3, consists in crossing females homozygous for third chromosomes of a given geographic locality to males which carry a *Bl Sc* chromosome, and another chromosome of a different geographic origin. This is, consequently, the repetition

of the type of cross shown in figure 1. However, in the offspring, wild-type females which carried chromosomes of different geographic origins were selected and outcrossed to males heterozygous for the *Bl Sc* and another third chromosome of the same locality as in the mother (figs. 2 and 3). The F_2 generation consists, then, of wild-type flies which carry one chromosome which may be a product of crossing over, and another chromosome which is of definite geographic origin. The *Bl Sc* flies in this generation have a *Bl Sc* chromosome, and a chromosome which may be a result of recombination. The *Bl Sc* males from the F_2 generation are now outcrossed in two ways. In the experiments referred to as " A-type " (fig. 2), they are mated to females which have two third chromosomes of the same origin. The offspring consists, then, of wild-type flies which have one third chromosome of geographically pure

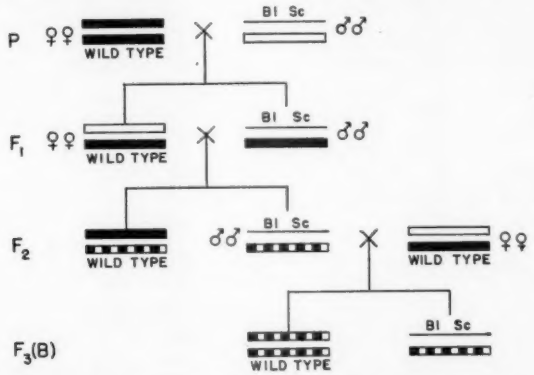


FIGURE 3.—Experiments designed to test the effect of crossing over between chromosomes of different geographic origin (shown in black and white) on the viability. Flies with two chromosomes of a pair resulting from crossing over (F_3(B)) are compared with flies having only one such chromosome (F_3).

and another of geographically mixed origin, and of *Bl Sc* flies which carry a *Bl Sc* and a geographically pure third chromosome. In the experiments of " B-type " (fig. 3), the *Bl Sc* males from the F_2 generation are crossed to females having two third chromosomes of different geographic origin. The offspring, then, consists of wild-type flies which carry two third chromosomes both of which may be crossover products, and of *Bl Sc* flies which carry a *Bl Sc* and a crossover third chromosome.

The results of the A and B types of crosses are summarized in table 3 (flies with chromosomes other than the third of Mather origin) and in table 4 (individuals with the genetic background of Gunnison origin). The data permit a number of instructive comparisons. First, the proportions of the wild-type class in the F_1 generation is compared with those obtained in control experiments in which the wild-type flies carried both third chromosomes of the same geographic origin. There are twelve such comparisons in tables 3 and 4, and

TABLE 3

Comparative viability of wild type flies in P, F_1, F_2, $F_3(A)$ and $F_3(B)$ in crosses with the Mather genetic background.

Crosses		Total flies	Percent Wild type	Chi square between the homozygotes and F_1 and $F_3(A)$	Chi square between F_1 and $F_3(A)$ and F_2 and $F_3(B)$
Mather / Mather × *Bl Sc* / Mather		1667	48.89		
Mather / Mather × *Bl Sc* / Bryce	F_1	1560	52.94	5.40 (P = 0.02)	
	$F_3(A)$	1018	44.99	3.96 (P = 0.05)	15.58 (P = <0.001)
	F_2	1273	45.87		
	$F_3(B)$	1601	44.28		0.68 (P = 0.50)
Mather / Mather × *Bl Sc* / Gunnison	F_1	1381	50.61	0.89 (P = 0.35)	
	$F_3(A)$	1243	47.54	0.56 (P = 0.50)	2.44 (P = 0.12)
	F_2	1310	47.70		
	$F_3(B)$	1540	46.29		0.56 (P = 0.50)
Bryce / Bryce × *Bl Sc* / Bryce		1688	48.63		
Bryce / Bryce × *Bl Sc* / Mather	F_1	1273	54.67	10.89 (P = <0.001)	
	$F_3(A)$	1095	46.57	1.17 (P = 0.30)	15.66 (P = <0.001)
	F_2	1295	49.11		
	$F_3(B)$	1377	42.70		11.15 (P = <0.001)
Bryce / Bryce × *Bl Sc* / Gunnison	F_1	1606	51.93	3.54 (P = 0.06)	
	$F_3(A)$	1049	47.47	0.39 (P = 0.55)	4.94 (P = 0.03)
	F_2	1254	48.24		
	$F_3(B)$	1234	46.59		0.64 (P = 0.50)
Gunnison / Gunnison × *Bl Sc* / Gunnison		1723	49.44		
Gunnison / Gunnison × *Bl Sc* / Mather	F_1	1602	50.81	0.58 (P = 0.50)	
	$F_3(A)$	1421	49.26	0.002 (P = 0.95)	0.76 (P = 0.45)
	F_2	1412	50.21		
	$F_3(B)$	1522	50.98		0.19 (P = 0.70)
Gunnison / Gunnison × *Bl Sc* / Bryce	F_1	1774	55.01	11.01 (P = <0.001)	
	$F_3(A)$	1441	49.82	0.04 (P = 0.90)	8.47 (P = 0.003)
	F_2	1381	46.12		
	$F_3(B)$	1404	47.01		0.20 (P = 0.70)

in every case the viability of the flies having two third chromosomes of different geographic origin is superior to that of the F_1 flies with the two chromosomes of the same origin. In eight of the twelve cases the differences are statistically significant. This agrees with the results of the experiments reported above (tables 1 and 2).

Another comparison is between the wild-type flies obtained in the F_3 of the A-type crosses and the control wild-type, which carries two chromosomes of the same origin. There are twelve comparisons of this kind in tables 3 and 4. In no case do the two kinds of wild-type flies show a significant difference in viability. The conclusion is quite different, however, if the wild-type flies of the F_3 (A) class are compared with those obtained in the F_1 generation (fig. 2). Among the twelve comparisons of this kind in tables 3 and 4, we have a lower viability in the F_3 flies in every instance, and in six of these comparisons the deterioration is statistically fully significant. The conclusion that fol-

lows is that the flies which carry two whole third chromosomes of different geographic origin are more viable than those in which one of the chromosomes is a mixture of parts of different geographic origin. This is confirmed by the fourth class of comparisons. These involve the wild-type flies from the F_3 (B) crosses and their wild-type parents from the F_2 (fig. 3). Here the F_3 (B) flies have two third chromosomes geographically mixed in origin, while the F_2 flies

TABLE 4

Comparative viability of wild type flies in P, F_1, F_2, F_3(A) and F_3(B) in crosses with the Gunnison genetic background.

Crosses		Total flies	Percent Wild type	Chi square between the homozygotes and F_1 and F_3(A)	Chi square between F_1 and F_3(A) and F_2 and F_3(B)
Gunnison / Gunnison × *Bl Sc* / Gunnison		1616	47.95		
Gunnison / Gunnison × *Bl Sc* / Mather	F_1	1323	52.08	4.98 (P = 0.02)	
	F_3(A)	1361	51.35	3.38 (P = 0.07)	0.14 (P = 0.70)
	F_2	1137	50.74		
	F_3(B)	1239	47.37		2.69 (P = 0.10)
Gunnison / Gunnison × *Bl Sc* / Bryce	F_1	1525	52.19	5.55 (P = 0.02)	
	F_3(A)	1382	48.69	0.93 (P = 0.35)	3.44 (P = 0.06)
	F_2	1033	44.43		
	F_3(B)	1407	42.64		0.82 (P = 0.45)
Mather / Mather × *Bl Sc* / Mather		1314	48.70		
Mather / Mather × *Bl Sc* / Bryce	F_1	1523	52.59	4.13 (P = 0.04)	
	F_3(A)	1473	49.08	0.05 (P = 0.85)	3.61 (P = 0.06)
	F_2	1141	48.81		
	F_3(B)	1320	46.13		1.67 (P = 0.20)
Mather / Mather × *Bl Sc* / Gunnison	F_1	1404	54.05	7.64 (P = 0.006)	
	F_3(A)	1254	46.33	1.40 (P = 0.25)	15.70 (P = <0.001)
	F_2	1721	49.68		
	F_3(B)	1235	41.94		17.53 (P = <0.001)
Bryce / Bryce × *Bl Sc* / Bryce		1492	49.66		
Bryce / Bryce × *Bl Sc* / Mather	F_1	1554	55.34	9.74 (P = 0.002)	
	F_3(A)	1271	46.65	2.57 (P = 0.15)	21.30 (P = <0.001)
	F_2	1340	49.40		
	F_3(B)	1327	45.21		4.71 (P = 0.03)
Bryce / Bryce × *Bl Sc* / Gunnison	F_1	1389	51.97	1.60 (P = 0.20)	
	F_3(A)	1584	48.98	0.13 (P = 0.70)	2.61 (P = 0.10)
	F_2	1495	50.16		
	F_3(B)	1514	44.25		10.32 (P = <0.001)

have only one such chromosome. Of the twelve such comparisons in tables 3 and 4, four show significant losses of the viability in the F_3 (B) flies, six show losses which do not reach the conventional level of significance, and two show insignificant increases.

I should also be pointed out that the flies having two crossover chromosomes, the F_3 (B) wild-type class, seem to be the least viable class in ten out of twelve series of the crosses recorded in tables 3 and 4.

DISCUSSION

Recent genetic work has shown that several distinct phenomena are confused under the common name of "heterosis." Populations of Drosophila and other sexually reproducing organisms carry in the heterozygous condition numerous recessive mutants and gene combinations which are deleterious, or even lethal, when homozygous. Inbreeding renders these deleterious genes homozygous, while crossing the inbred lines restores the dominance of the "normal" alleles tested by natural selection (mutational heterosis). On the other hand, as pointed out particularly by CROW (1948, 1952), heterosis may arise through interaction of alleles, or gene complexes, all of which are deleterious in homozygous condition. Natural selection maintains such alleles and gene complexes in populations, leading to the state of balanced polymorphism. The greater the variety of such heterotic genes the smaller the proportion of individuals homozygous for them in the population. Inbreeding increases the frequency of homozygosis, crossing the inbred lines restores the "normal" reproductive biology (the balance heterosis). By far the best evidence of the prevalence of balanced polymorphism in natural populations comes from observations and experiments on the chromosomal polymorphism in *Drosophila pseudoobscura* and other species (DOBZHANSKY 1951).

A still different type of heterosis is indicated by the results of VETUKHIV (1953), who worked with the strains of *Drosophila pseudoobscura* from the same localities which were used in the experiments described in the present paper. VETUKHIV observed that crossing of different geographic populations leads to an increase of the viability in the F_1 hybrids. Since the populations crossed are not inbred, they must be presumed to possess their normal heterotic state. This being the case, the further increase of the viability in F_1 hybrids suggests that heterozygosis may, up to a certain point, enhance the viability of the heterozygotes.

The results reported in the present article confirm and extend those of VETUKHIV. It is clear from the data in tables 1–4, that the presence of a single chromosome transferred from another geographic population of the same species increases the vigor of its carriers. Since such heterozygotes do not occur in natural populations, except perhaps very rarely, one can hardly suppose that the gene complexes in the chromosomes which occur in one geographic race are coadapted by natural selection to produce superior vigor in combinations with chromosomes of other races. A more direct effect of heterozygosity seems to be an assumption necessitated by the data.

The increased vigor in the F_1 hybrids contrasts, however, with a breakdown of the vigor as a result of gene recombination in the F_2 generation. VETUKHIV (1953) has observed such a breakdown in the F_2 hybrids from his crosses. The data reported in the present paper again confirm and extend his results. When a chromosome transferred from a foreign population is broken up by crossing over with chromosomes of different geographic origin, the resulting crossover products do not give a superior vigor to their carriers. In fact, the

F_2 and F_3 hybrids are inferior not only to the F_1, but in most cases even to the parental strains themselves (tables 3 and 4). The favorable effects of the presence of many heterozygous loci are more than offset by the disintegration of the internally balanced combinations of genes carried in the chromosomes of every population.

The existence of such internally balanced gene systems in the chromosomes in natural and certain experimental populations has been postulated by many authors (MATHER 1943; MATHER and HARRISON 1949; DOBZHANSKY 1949; DOBZHANSKY and PAVLOVSKY 1953; WALLACE 1952; WALLACE et al. 1953a, 1953b). Specialized chromosomal mechanisms, such as inversions found in many natural populations, have as their biological function the preservation of such balanced gene systems. The integrity of the gene system in a chromosome is easily lost by crossing over with chromosomes from foreign populations. It may be pointed out in this connection that the gene complex carried in chromosomes with the Arrowhead gene arrangement in the Mather population have become established in nature because of the high adaptive value of the heterozygotes formed with other chromosomes in the same population. At Mono, Lehman, Bryce and Ferron most of the flies are homozygous for Arrowhead chromosomes, while at Gunnison, the chromosomes with the Pikes Peak gene arrangement become common (DOBZHANSKY 1951). The "viability stimulus" found in the F_1 hybrids between different populations is definitely lost in the later generations. The lowest viability is encountered in individuals in which both chromosomes of a pair are crossover products, as in the F_3 (B) flies shown in figure 3 and in tables 3 and 4.

ACKNOWLEDGMENTS

The writer is deeply indebted to PROFESSOR TH. DOBZHANSKY for his hospitality, for the guidance and valuable advice during the work, and his help in the preparation of the manuscript. The author also wishes to thank MR. BORIS SPASSKY for generously furnishing the stocks used in the investigation, and to PROFESSORS J. T. PATTERSON and W. S. STONE for their suggestions and for extending the facilities of the laboratories of the Department of Zoology at the University of Texas.

SUMMARY

The purpose of the experiments reported in the present article has been to study the integration of the genotype of local natural populations of *Drosophila pseudoobscura* in the absence of variations in the gene arrangements (inversions). The material used consisted of strains of *D. pseudoobscura* from several localities in California, Nevada, Utah and Colorado. All strains were homozygous for the Arrowhead gene arrangement in the third chromosomes. By means of a series of crosses, executed by MR. B. SPASSKY, third chromosomes from these various localities have been transferred to the genotypic backgrounds characteristic of the Mather, California, and of Gunnison, Colorado local populations.

F_1 hybrids, which carry two third chromosomes of different geographic origin are more viable than the flies with two third chromosomes from the same geographic locality. In F_2 and F_3, a breakdown of the viability is observed, and in most cases the viability is inferior to that in the original parents.

In the later experiments it has been possible to demonstrate that this breakdown of heterosis in F_2 and F_3 is an effect of the recombination by crossing over between the chromosomes derived from different populations. The lowest viability has been found in individuals in which both chromosomes of a pair are crossover products.

The general conclusion is that an internally balanced gene complex is maintained in every geographic population by natural selection. These complexes suffer disintegration owing to recombination and crossing over in the progeny of the hybrids.

LITERATURE CITED

CROW, J. F., 1948 Alternative hypotheses of hybrid vigor. Genetics **33**: 473–487.

1952 Dominance and overdominance. Heterosis, edited by J. W. Gowen. Iowa State College Press: 282–297.

DOBZHANSKY, TH., 1948 Genetics of natural populations. XVIII. Experiments on chromosomes of *D. pseudoobscura* from different geographic regions. Genetics **33**: 588–602.

1948 Observations and experiments on natural selection in Drosophila. Hereditas, Suppl.: 210–224.

1951 Genetics and the origin of species. 3rd Ed. Columbia University Press, New York.

DOBZHANSKY, TH., and C. EPLING, 1944 Contributions to the genetics, taxonomy, and ecology of *D. pseudoobscura* and its relatives. Carnegie Inst. Publ., Washington, D. C. **554**: 1–183.

DOBZHANSKY, TH., and O. PAVLOVSKY, 1953 Indeterminate outcome of certain experiments on Drosophila populations. Evolution **7**: 198–210.

MATHER, K., 1943 Polygenic inheritance and natural selection. Biol. Rev. **18**: 32–64.

MATHER, K., and B. J. HARRISON, 1949 The manifold effect of selection. Heredity **3**: 1–52 and 131–162.

VETUKHIV, M., 1953 Viability of hybrids between local populations of *Drosophila pseudoobscura*. Proc. Nat. Acad. Sci. **39**: 30–34.

WALLACE, B., 1952 A genetic analysis of the adaptive values of populations. Proc. Nat. Acad. Sci. **38**: 700–715.

WALLACE, B., and C. V. MADDEN, 1953a The frequencies of sub- and supervitals in experimental populations of *Drosophila melanogaster*. Genetics **38**: 456–470.

WALLACE, B., J. C. KING, C. V. MADDEN, B. KAUFMANN, and E. C. MCGUNNIGLE, 1953b An analysis of variation arising through recombination. Genetics **38**: 272–307.

NATURAL SELECTION IN CEPAEA

A. J. CAIN
Department of Zoology and Comparative Anatomy, University of Oxford

P. M. SHEPPARD
Genetics Laboratory, Department of Zoology, University of Oxford

Received May 29, 1953

THE remarkable variation in colour and ornamentation of the shell which is shown by certain gastropods has attracted the attention of collectors for many years. Recently, several studies of this variation have appeared, which unfortunately lead to very diverse conclusions on its mechanism of maintenance and its significance in evolution. One of the commonest and most strikingly variable snails of Europe is *Cepaea nemoralis,* which has been studied in considerable detail by several workers, mainly in Europe but also in the U.S.A. where it has been successfully introduced. DIVER (1940) has concluded that its range of variation is wholly random, BOETTGER (1931) that on the contrary it is determined by natural selection, while LAMOTTE (1951) believes that for banding, at least, it is maintained in each colony primarily by mutation, and SCHILDER (1950) by gene flow.

Our own investigations (CAIN and SHEPPARD 1950, 1952; SHEPPARD 1951, 1952) lead us to the conclusion that variation is maintained by balanced polymorphism (FISHER 1930a; FORD 1940), but the proportions of different varieties in each colony are strongly affected by selective visual predation. In this paper the principal studies on *Cepaea nemoralis* are examined, and further evidence for the importance of natural selection is presented.

SYSTEMATICS

The genus Cepaea Held 1837, proposed and still often ranked as a subgenus of Helix L., contains four very closely allied species, reviewed by PERROT and PERROT (1938). *C. vindobonensis* is confined to southeastern Europe and the Caucasus, *C. sylvatica* to the western Alps. *C. hortensis* ranges through north, central and western Europe from Finland to the Pyrenees, and the British archipelago, and as far as Iceland and northeastern North America. *C. nemoralis* is confined to central and western Europe from northern Italy (LAMOTTE 1951, p. 45) and Czechoslovakia to the Pyrenees, Ireland, south Scotland and southern Scandinavia. It has been introduced into Canada, the United States, and New Zealand. All these species overlap geographically except the closely allied *C. sylvatica* and *vindobonensis,* but *sylvatica* is a high mountain form, probably isolated by altitude from all the others, according to the data given by LAMOTTE (1951). *C. sylvatica* and *vindobonensis* both have a haploid chromosome number n = 25, *C. hortensis* and *nemoralis* have n = 22 (PERROT and PERROT 1938). Hybridization between *C. nemoralis* and *hortensis* has been

well reviewed by LAMOTTE (1951). It is extremely rare in the wild. Hybrids have been produced with some difficulty in the laboratory (LANG 1904, 1912; KLEINER 1912). DIVER (1940) observed courtship between individuals of *hortensis* and *nemoralis* and concluded that specific differences in courtship behaviour probably formed an efficient barrier to fertilization.

POLYMORPHISM

Although all the species of Cepaea are variable in shell characters, *C. nemoralis* is particularly so. The lip is normally black or dark brown, rarely pink or white. The rest of the shell may be yellow, brown or any shade from very pale fawn through pink and orange to red. Up to five black or dark brown (seldom transparent) longitudinal bands may be present, very rarely more. All possible combinations of presence or absence and of fusion of adjacent bands have been recorded (TAYLOR 1914), but their frequencies vary considerably; particularly common are five-banded, and unbanded shells and those with only the middle band. For recording, the bands are numbered 1 to 5, the uppermost being 1. Fusions are indicated by bracketing the numbers representing the fused bands, absence by 0. This system is slightly simplified from that proposed by VON MARTENS (see LAMOTTE 1951). The bands may vary greatly in width, and also in their point of origin along the shell. A band may be absent or nearly absent either because it has been reduced in width to the vanishing point, or because its formation has been postponed until the animal has nearly or quite stopped growing. Fusions also may occur at any time after the appearance of the bands affected. In his monographic study of variation in *C. nemoralis*, LAMOTTE (1951) classifies the banding varieties by the presence or absence of bands, ignoring fusions. Apparently, a band is counted as present even if reduced to a mere trace near the lip. In our papers, these traces if appearing only for one-fourth inch or less and only close to the lip, are not recorded as present, since they are phenotypically almost insignificant. Fusions are recorded only if they occupy a large part of the largest whorl of the shell (CAIN and SHEPPARD 1950).

Although most of the various banding-varieties grade into each other because of variation in the development and fusion of bands, some appear clear-cut. The unbanded form 00000, and that with only the median band, 00300, are usually distinct from the five-banded ones (12345 with or without fusion), intermediates with more than the slightest traces being very rare. On the other hand, every intermediate due to reduction in width of bands between 12345, 02345, 10345, 00345 and 00045 may occur in the same colony. In some colonies, however, certain of these combinations may occur without intermediates. The intensity of pigmentation of the bands varies somewhat; rarely there occurs a very distinctive variety in which there is no dark pigmentation. Although the ground colour is present, these individuals are referred to in the literature as albinos. The bands are transparent and tinged with the ground colour of the shell, and the lip is white or pink.

There are three distinct colour classes. The calcareous layers of the shell are

suffused with yellow, pink, or dull violet which in combination with the yellow proteinous periostracum produce respectively yellow, a range of colours from fawn to red, and dull brown. LAMOTTE (1951) appears not to have found examples of the last class.

In almost every colony, at least two colour and banding classes are represented, and frequently many more. The compositions of many French colonies are given by LAMOTTE (1951) and of some English ones by us (1950, 1952).

GENETICS

The earlier work on Cepaea has been admirably reviewed by LAMOTTE (1951) who has added considerably to the available information. It is certain that yellow ground colour is recessive to pink. The genetics of brown are not known. In our experience it is a clear-cut character almost certainly controlled by a single factor (see p. 96). The unbanded form 00000 is dominant to both 12345 and to 00300 which itself is dominant to 12345. It is highly probable, although not proved, that 00300 is not an allelomorph of 12345. LANG (1912) for *C. nemoralis* and BOETTGER (1950a) for *C. hortensis* have suggested dominance relations for 00345 and 00045, but do not give the necessary data. The character " pale or interrupted bands " as against the normal darkly pigmented and continuous ones has been studied by LANG (1908, 1912) and by LAMOTTE (1951) whose data support LANG's suggestion that it is controlled by a dominant gene, which is not an allele of colour or the various banding patterns. The character albino is recessive to normal pigmentation (of the lip and of bands when present). We can find no data to show whether it is an allele of " pale bands " or not: it is certainly not allelic with colour or banding patterns.

FISHER and DIVER (1934) have shown linkage between colour and banding with a crossover value of approximately 20%. STELFOX (unpublished data quoted by them) found the same linkage but with a crossover value significantly less than FISHER and DIVER's: in fact, in his experiment he observed no crossing over. Pink and yellow, and unbanded and some unspecified banding variety were investigated. FISHER and DIVER suggest that either the factors for pink were not the same in their and STELFOX's experiments, or that the closeness of linkage varies; presumably they and STELFOX used the same banding variety, otherwise this also could have differed. PROFESSOR J. B. S. HALDANE has suggested to us that the differences in crossover value may be associated with inversions. Unfortunately the chromosomes (figured by PERROT and PERROT 1938) are not good material for investigating this cytologically.

Linkage cannot normally be demonstrated from random samples of wild populations, but if it is very close, or there is selection for certain linked combinations, it can be observed. A striking deficiency of certain combinations without linkage could be brought about only by extremely heavy selection or by nonrandom mating. After extensive experiments, LAMOTTE (1951) has been unable to find any evidence of mating nonrandom with respect to colour and banding varieties of *C. nemoralis*. The data of SCHNETTER (1950) and SCHILDER (1926 and 1950) confirm this conclusion. DIVER (1932) has already

TABLE 1

Albinos

Locality		Yellow			Pink			Brown			Totals	Total
		00000	00300	Others	00000	00300	Others	00000	00300	Others		
Ballyness Bay	Normal	35	0	29	0	0	0	0	0	0	64	72
	Albino	0	0	8	0	0	0	0	0	0	8	
Thruxton	Normal	0	0	0	2	86	10	24	5	0	127	156
	Albino	0	0	0	0	29	0	0	0	0	29	
Pentridge I	Normal	0	63	196	1	34	93	6	2	18	413	428
	Albino	0	3	12	0	0	0	0	0	0	15	
Pentridge II	Normal	0	24	43	2	17	46	1	3	5	141	150
	Albino	0	3	6	0	0	0	0	0	0	9	

pointed out that linkage can be observed in wild populations of *C. nemoralis* and states (unfortunately without giving data) that all the shell characters investigated by him are linked. Our data tend to support this view, as will be seen from tables 1–6.

TABLE 2

Locality	Yellow			Pink			Brown			Total	Effectively unbanded except 00000 and 00300	00000 with traces in samples of more than 50
	00000	00300	Others	00000	00300	Others	00000	00300	Others			
Beechwoods												
Ashbury	0	0	3	5	1	5	17	0	0	31	0	....
Great Beech Wood	1	1	0	34	23	22	19	0	0	100	2	0
Beechgrove Farm Wood	0	0	5	31	0	16	7	0	0	59	0	1
Great Bear Wood	0	1	1	4	5	7	3	0	0	21	0	....
Pulpit Hill (top)	0	0	2	29	0	4	0	0	0	35	0	....
Rockley 1	1	5	0	4	1	0	8	1	1	21	0	....
Rockley 2	18	16	0	51	7	0	45	2	0	139	0	0
Rockley 6	9	4	0	8	19	0	43	0	0	83	0	0
Rockley 8	2	4	0	6	4	1	12	1	0	30	0	....
Rockley 9	1	3	0	2	6	1	9	3	0	25	1	....
Hackpen	15	24	0	78	39	0	1	1	0	158	0	18
Aston Rowant	0	0	0	23	0	7	0	0	0	30	1	....
Oakwoods												
Great Bedwyn Wood	1	2	0	3	13	0	2	0	0	21	0	....
Putall Gate	9	7	8	146	188	211	62	0	1	632	7	51
Boarstall Wood	0	0	0	54	28	51	0	0	0	133	0	6
Oakley Wood	0	2	4	8	41	72	0	0	0	127	3	7
Tackley Heath Wood	0	28	32	79	51	60	0	0	0	250	4	0
Stanton St. John Gt. Wood	0	0	0	5	7	10	0	0	0	22	0	....
Hell Coppice	0	2	0	10	27	16	3	0	0	58	0	1
Cobham Frith	0	25	1	54	88	25	0	4	2	199	0	24
Broomsgrove	1	4	5	5	30	5	30	15	2	97	0	12

It is not always possible to distinguish between a nonrandom distribution of characters due to linkage, and to multiple allelomorphs. Furthermore if the expression of an allele at locus A is dependent on the presence of a particular allele at locus B, and B is linked to a third locus, C, but not to A, nevertheless A may appear to be linked to C. This situation also cannot always be identified

TABLE 3

Locality	Yellow			Pink			Brown			Total	Effectively unbanded except 00000 and 00300	00000 with traces in samples of more than 50
	00000	00300	Others	00000	00300	Others	00000	00300	Others			
Mixed deciduous woods												
Filchamstead Wood	0	19	9	21	59	23	8	0	0	139	0	1
Pusey Common Wood	1	0	19	45	1	31	69	0	0	166	0	0
Little Wittenham Wood	3	0	9	69	12	67	63	0	0	223	3	13
Burdrop Wood	1	5	38	17	3	23	23	1	0	111	5	16
Wantage 1	2	0	7	13	0	2	3	0	0	27	0	....
Wantage 2	7	0	7	7	0	23	8	0	0	52	0	3
My Lady's Seat, Wytham	39	14	117	91	31	217	105	0	0	614	143	2
Hen Wood	5	34	37	2	47	80	0	0	0	205	7	0
Wytham Hill Top	20	8	28	30	17	68	17	0	0	188	16	0
Spindle Trees, Wytham	11	15	28	31	51	120	34	0	0	290	45	5
Reserve Ride, Wytham	15	7	18	39	35	108	17	0	0	239	23	0
Lord's Common, Wytham	1	4	32	3	15	69	20	0	5	149	25	2
Streatley South Wood	2	4	11	84	11	33	17	0	0	162	0	24
Marley Wood, Wytham	4	74	329	210	146	662	83	0	0	1508	158	2
Common Piece Spruces, Wytham	9	19	42	94	87	197	14	0	0	462	40	2
Rockley Copse Cumnor	0	1	10	10	4	71	8	0	0	104	9	3
Marley Bog, Wytham	4	25	91	91	69	221	58	0	1	560	49	3
Bagley Wood	0	8	23	19	26	72	19	0	0	167	2	7
Blackwater Wood	0	1	3	7	5	18	1	0	0	35	0	....
Stanton Little Wood	0	4	3	3	2	12	0	0	0	24	0	....
Gt, Copse, Chislehampton	106	0	65	3	1	18	21	0	0	214	1	0

from such data. We will therefore refer to nonrandom distribution due to any of these situations as *associations* between the characters concerned.

In table 1 are shown the only colonies we have found in which albinism is sufficiently frequent for association to be investigated. No unbanded albinos appear in it, but they have been found by us, and are reported by BOETTGER (1931) and LAMOTTE (1951). It is clear from the table that albinism is associated with colour and with the 00000 locus.

TABLE 4

Locality	Yellow			Pink			Brown			Total	Effectively unbanded except 00000 and 00300	00000 with traces in samples of more than 50
	00000	00300	Others	00000	00300	Others	00000	00300	Others			
Hedgerows												
Little Wittenham	11	1	43	6	0	54	17	0	1	133	2	0
University Farm	31	16	92	18	0	33	5	0	0	195	21	0
Bletchingdon Road	2	2	8	3	0	22	1	0	0	38	3	....
Shrivenham	0	1	93	1	0	16	9	0	0	120	1	0
West Adderbury Turn	33	24	68	37	25	44	10	0	0	241	1	0
Culham Hedge	0	22	18	0	9	40	20	0	0	109	0	0
Tackley Turn	0	4	224	0	8	82	0	0	0	318	10	0
Banbury-Warmington	13	28	33	7	23	29	0	0	0	133	1	0
Tackley Heath	1	13	67	0	11	67	0	0	0	159	5	0
Hedge nr. Tackley Turn	1	8	139	0	4	43	5	0	0	200	52	1
Eynsham Road	36	5	81	2	4	22	2	0	0	152	3	0
Easington	1	2	12	1	1	8	0	0	0	25	0	....
Near Wroxton	2	4	6	3	3	19	0	0	0	37	0	....
Edge Hill	4	2	10	0	0	1	0	0	0	17	0	....
Fyfield	29	4	26	22	11	59	0	0	0	151	3	0
Faringdon Road	3	21	61	0	4	23	0	0	0	112	7	0
East of Pusey Common Wood	2	0	6	2	0	8	0	0	0	18	0	....
Golden Butts	0	5	12	0	1	4	0	0	0	22	0	....
West of Pusey Common Wood	23	2	34	11	5	93	4	0	0	172	1	0
Oak Holt	0	15	38	3	9	34	0	0	0	99	0	0
Cumnor-Farmoor	2	5	72	0	1	32	22	0	0	134	2	2
Botley	1	0	37	0	0	4	15	0	0	57	6	0
Stretham	0	2	26	0	0	3	0	0	0	31	2	....
Kingston Hill	10	4	58	8	2	18	0	0	0	100	1	0
Parks Hedge	1	1	12	0	1	61	0	0	0	76	0	0
Canal Bank	17	25	72	7	27	120	9	1	1	279	10	0
Hinksey Hedge	5	2	11	4	2	10	0	0	0	34	1	....
Wytham Lane 1	2	1	13	1	0	5	0	0	1	23	1	....
Wytham Lane 2	3	0	23	0	0	17	11	0	0	54	7	0
Standlake	3	7	42	14	1	5	8	0	0	80	2	0
Woodstock Road	1	2	17	1	3	5	0	0	0	29	3	....
Hedge S. of Islip	3	12	33	4	9	22	4	0	0	87	1	0
Woodstock Rd. level crossing	8	6	14	0	0	0	0	0	0	28	0	....

In the Ballyness Bay collection there is a significant deficiency of unbanded albinos ($P < 0.01$). There is an association in the Thruxton collection but it is not possible to determine whether it is between albino and colour or albino and 00000 (P for pink vs. brown is < 0.01). The data from the Pentridge show that there is an association with colour ($P < 0.01$), and this is confirmed by the data from a separate collection from the Pentridge (Pentridge 2), which was not taken at random, the more brightly coloured shells being preferred.

But this preference merely raised the proportion of pink shells taken and therefore increased the probability of obtaining albino pinks if they were present. In fact, no albino pinks were found at all at this locality.

It appears from tables 2–6 that 00000 as against banding, but not 00300 as against other banded forms, is associated with all three colours. The association is too strong to be due to random sampling ($P < 0.001$). Absence of association in any colony does not mean absence of linkage: presence of appar-

TABLE 5

Locality	Yellow			Pink			Brown			Total	Effectively unbanded except 00000 and 00300	00000 with traces in samples of more than 50
	00000	00300	Others	00000	00300	Others	00000	00300	Others			
Rough herbage												
Wittenham Clumps	13	3	177	6	3	75	2	0	0	279	3	0
Wittenham Clumps downland	5	0	14	0	0	4	0	0	0	23	0	....
Hinksey Hill	59	42	267	5	4	57	0	0	0	434	32	0
Southmoor	69	19	106	34	1	27	2	0	0	258	7	0
Bicester Road	30	32	50	0	5	18	14	0	0	149	2	0
Nettlepatch, Cumnor Hill	19	0	50	39	0	53	0	0	0	161	11	0
Southern Bypass	25	13	58	11	7	53	0	0	0	167	21	0
Attleborough	0	0	108	0	0	64	0	0	0	172	0	0
Cutslow Park	4	1	30	1	1	15	1	0	0	53	2	0
Aston Tirrold	6	0	9	7	0	23	20	0	1	66	8	2
Scolt Head	3	10	137	0	0	2	4	0	14	170	3	0
Rugby	58	63	53	17	20	12	0	0	0	223	0	0
Sheepstead Hurst	1	3	22	4	1	20	7	0	0	58	0	0
Eynsham Tollbridge	0	2	62	0	1	45	0	0	0	110	2	0
Cassington Halt	30	32	68	0	10	7	0	0	0	147	9	0
Northern Bypass	5	2	31	0	8	20	7	0	0	73	4	0
Hurst Rise Road	3	8	52	1	1	14	2	0	0	81	3	0
Chilswell Farm	4	2	12	0	0	3	0	0	0	21	1	....
Christmas Common downland	0	11	55	19	6	61	0	0	0	152	18	1
Pentridge 1	0	66	208	1	34	93	6	2	18	428	4	2
Pans Hill	0	8	18	0	30	63	0	0	0	119	2	0
Kennington Road	7	1	12	6	2	5	1	0	0	34	0	....

ent association in only one or two among a large number of colonies could arise from sampling errors. The data from colonies in Germany and Austria given by SCHILDER (1926) also indicate linkage between colour and 00000. The presence of these striking associations can be due only to exceedingly heavy selection, or to a moderate degree of linkage with selection, or to very close linkage indeed, or to multiple allelomorphism. Very heavy selection of the degree necessary to produce these striking associations without linkage is most unlikely. Multiple allelomorphism does not appear to be extensive. Albino

is clearly not an allelomorph of colour or banding; it is linked to banding and to colour. 00000 is associated with all three colours and has been shown by FISHER and DIVER (1934) to be linked with the pink-yellow locus. Brown could be an allelomorph of yellow and pink or a different gene converting either or both of the other colours to brown. If it be a separate gene, it is certainly linked with 00000, which itself is linked with the pink-yellow locus. If it converts only one of the other colours, the ratios of banding classes in the brown class should be the same as in either pink or yellow. The tables show

TABLE 6

Locality	Yellow			Pink			Brown			Total	Effectively unbanded except 00000 and 00300	00000 with traces in samples of more than 50
	00000	00300	Others	00000	00300	Others	00000	00300	Others			
Short turf												
Crowell Hill	0	0	32	13	0	95	0	0	0	140	12	0
Rockley 5	58	55	1	27	11	0	64	2	0	218	1	0
Rockley 7	7	7	1	4	3	0	11	1	0	34	1	....
Rough Down	119	35	12	7	23	13	93	16	6	324	12	7
Kimpton Down	0	1	12	18	20	15	10	9	9	94	1	2
Thruxton	0	0	0	2	115	10	24	5	0	156	2	2
Background unscorable												
East Ilsley	0	0	6	0	1	0	27	4	4	42	2	....
Rough Common, Wytham	12	7	37	20	12	85	13	0	0	186	14	0
Sowberry Court	2	0	9	15	0	27	36	0	0	89	0	14
Chute Windbreak	1	6	5	1	12	13	0	1	0	39	0	....
Pulpit Hill (Lower slope)	0	3	37	52	3	59	0	0	0	154	7	0
Cothill	0	0	16	5	1	11	0	0	0	33	3	....
Colonsay	0	3	33	0	1	35	0	0	0	72	0	0
Sliding Hill	6	3	17	27	6	43	0	0	0	102	1	1
Rockley 4	22	17	0	19	10	0	42	0	0	110	0	0
Rockley 3	18	12	0	22	4	0	21	0	0	77	0	1
Shepherd's Hey	1	0	31	13	0	21	5	0	0	71	2	0
Skegness	0	0	14	0	0	18	4	0	0	36	0	....

clearly that this is not so. Brown must therefore be either an allelomorph at the pink-yellow locus or linked with it.

There is evidence that 00300 is at a different locus from 00000 (LAMOTTE 1951) and our data do not show that 00300 is associated with colour. Some of LAMOTTE's data for French colonies suggest linkage at the other loci.

It seems, then, that certainly three and probably five loci are involved in the production of the commonest variations, and that there are at least three very closely linked genes associated with the polymorphism. They are 00000, Albino and Pink together with their allelomorphs.

SELECTION

Several authors (HALDANE 1932; ROBSON and RICHARDS 1936; DIVER 1940; DOBZHANSKY 1941 but not 1951; HUXLEY 1942; MAYR 1942; CARTER 1951) have stated that variation in *C. nemoralis* is random; some have suggested that the differences between colonies or parts of colonies are very probably due to genetic drift. These conclusions rest upon an error of procedure (CAIN 1951a, b). Because a worker has been unable to correlate variation with any environmental or other factors, it does not follow that no correlations exist. In fact, the distribution of varieties in this species is controlled primarily by natural selection.

Selection by visual predation

Selection for both colour and banding has been directly demonstrated. SHEPPARD (1951) has shown that in Wytham Woods thrushes (*Turdus ericetorum*) do exert a visual selection while preying on these snails, and that the selection varies with seasonal changes in the background on which the snails live. When the woodland floor is brown with leaf-litter and exposed earth in early spring, yellow shells are at a disadvantage to brown or pink ones, but when in late spring the background becomes green, they are at an advantage. LAMOTTE (1951) has shown by the comparison of intact shells with shells broken by thrushes that in certain localities unbanded shells were at a disadvantage to five-banded ones. According to him, shells with 00300 or 00345 were intermediate in selective value but closer to the unbanded. We have investigated a colony of *C. nemoralis* in Marley Bog, a small hill-side fen, maintained by spring water, in Wytham Woods. The colony is predated by thrushes which are forced to carry the snails a few yards on to a bank above the fen where there are stones, in order to crack them. The thrushes were watched every day from July 4 to 20, 1951 by MR. J. A. GIBB, who very kindly collected the broken shells for us. Collections of live snails were made by us, taking all the snails discovered in an intensive search of a series of small patches of herbage: the composition of these is given in table 7. There was no

TABLE 7

	Unbanded shells	Other effectively unbanded shells	Effectively banded shells	Totals
Living snails	153	143	264	560
Predated snails	204	173	486	863

evidence of selection for colour, which is accordingly disregarded in this table, from which a comparison of live and predated snails can be made. There is good evidence ($P < 0.02$) that unbanded shells 00000 are at a selective advantage to effectively banded ones. In previous papers (CAIN and SHEPPARD 1950; SHEPPARD 1952) we have suggested that since the lower bands are normally partially or completely hidden in the living snail, shells with bands 1 and 2 both absent can be considered as effectively unbanded phenotypically. LA-

MOTTE's data suggest that the varieties 00300 and 00345 may be intermediate in selective value between 00000 and 12345, as might be expected. At Marley Bog, the selective value of 00300 and 00345 (which with 00000 make up the class of effectively unbanded shells there) is so close to that of 00000 that the figures give no indication whatever of any difference, but do show a significant difference of these two combined from effectively banded shells ($P < 0.001$). Consequently these forms at Marley Bog are effectively unbanded. Since in both LAMOTTE's and our data these intermediate forms tend to resemble 00000 in selective value, it is justifiable to combine them with 00000 in a class of effectively unbanded as against effectively banded shells, a useful simplification. Direct evidence of selection thus supports a classification originally based on the appearance of the shells judged by the human eye.

Direct evidence of selection has so far been obtained in a few localities only, but is amply supported by indirect evidence drawn from a large number of colonies. We have investigated all the colonies we have found, and our sample of them is biased only in so far as we have looked for these snails in localities where the background could be classified (as beechwoods, downland, hedgerows etc.), avoiding those where there is a mixture of types of vegetable formations. The few colonies on mixed background that have been found are listed separately in table 6.

It was shown (CAIN and SHEPPARD 1950) that there is a strong correlation between the proportions of varieties in each colony and the characteristics of the exact background on which the snails are living. On the greener backgrounds there is a high proportion of yellow shells, on the more uniform a high proportion of effectively unbanded ones. This result is completely confirmed by more extensive collecting. The data for all our samples (except a few very small ones listed in CAIN and SHEPPARD 1950) are set out in tables 2–6 and a correlation diagram for percentage yellow shells, percentage effectively unbanded, and habitat is given in figure 1. The samples from the Pentridge, Scolt Head, Skegness, Ballyness Bay and Colonsay are omitted from the diagram, since we have not collected them personally nor seen their localities. In general, the classification by habitat gives a measure of the smoothness and greenness of the background (CAIN and SHEPPARD 1950). It will be seen that colonies in the same habitat-class tend to group together in the diagram (most closely in the least variable habitats). But it should also be noticed that even when the habitat classification is disregarded, there is an extremely good correlation between percent yellow and percent effectively unbanded. Because colour and banding are known to be genetically distinct, and there is no constant association of yellow with any particular banding-formula in individuals, this in itself can only mean that selection is operating. We have shown that this correlation is chiefly due to an association of colour and uniformity in the backgrounds we have studied, the browner ones being usually the more uniform.

In several of the colonies on mixed backgrounds, the background can be partially classified. Those at Sowberry Court and East Ilsley have already been discussed (CAIN and SHEPPARD 1950). Rough Common (Wytham) is partly mixed deciduous woodland and partly open grassland. The colony at Chute

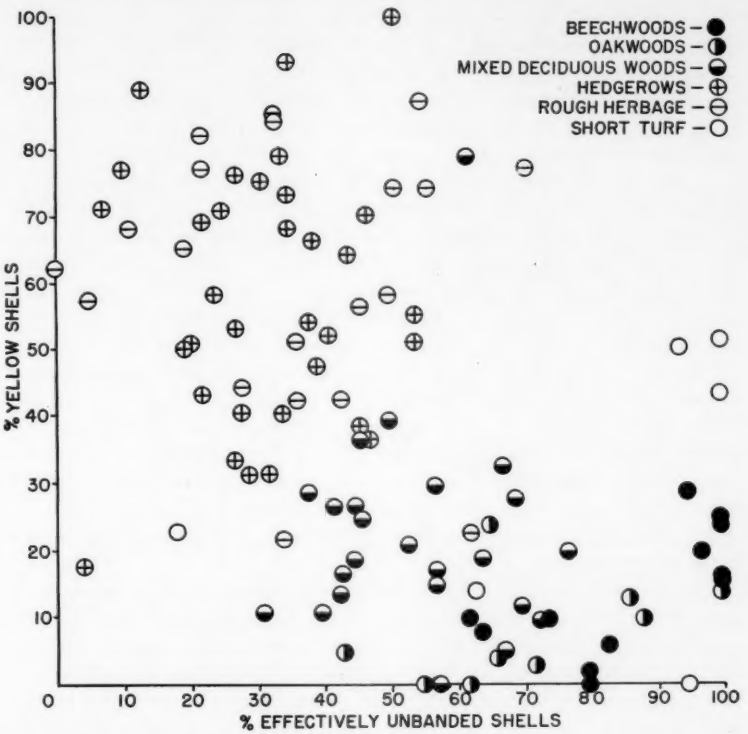


FIGURE 1.—Correlation-diagram, for % yellow shells, % effectively unbanded shells, and habitat, of all colonies of *Cepaea nemoralis* seen by us, except those with background unscorable.

windbreak was in a belt of beechwood 20 yards wide and in adjacent rough herbage. That at Pulpit Hill was on the lower slopes of a chalk escarpment, with scattered beech and oak trees, hawthorn and elder bushes, rough herbage, and patches of smooth green grass. In general the habitat approximates to woodland. Cothill comprises grassland, rough herbage and mixed deciduous woodland. The sample from Sliding Hill was from a mixture of habitats much as at Pulpit Hill but with ash instead of oak, and no hawthorn. Those from Rockley 3 and 4 are described by SHEPPARD (1952). That at Shepherd's Hey was from beechwood with much grass and a ditch full of wind-driven beech leaves bordered by a hedge of hawthorn with brambles. In all these colonies the proportions of varieties agree with their backgrounds taken as a whole. The localities most like woodland (Sowberry Court, Pulpit Hill, Sliding Hill, Shepherd's Hey) have colonies resembling those in the woodland classes, the others tending to be intermediate between the characteristics expected for the extreme types of habitat present.

Six colonies appear to have characteristics exceptional for the habitat class. Two, at Pan's Hill and Great Copse, Chislehampton, are in complete agreement with the backgrounds, which are exceptional in their habitat-classes. The colony at Pan's Hill is in a large thicket of rosebay willowherb merging with dense brambles. Although the habitat must be classified as rough herbage, the background at and just above ground level is brown, not green: correspondingly, the proportion of yellows is very low. Great Copse is an open elm wood on a hillside, with several springs, and the background at ground-level is of brilliant green short grass. The proportion of yellow is very high. Out of a total of 104 localities investigated by us personally, only four are really exceptional; these are at Crowell Hill, Aston Tirrold, Thruxton, and Kimpton Down, and, remarkably enough, they have many characters in common. All are on steep downland slopes with much grass, and a few bushes. All are infested by rabbits, and most of the shells collected were rabbit-predated. All have an unexpectedly low proportion of yellows, considering the greenness of the background, and at Crowell Hill, there is an unexpectedly high proportion of banded shells, much of the turf there being smooth and rabbit-grazed. All have a high population-density of *C. nemoralis*. The strong similarity of these four exceptional colonies indicates that there is some definite reason for their peculiarities other than mere random variation. It is possible (CAIN, in press) that rabbit predation by tone only, not colour and tone, is of particular importance in these four localities.

The similarity between colonies in similar habitats cannot possibly be due either to migration between adjacent colonies, or to geographical variation correlated with broad features of the environment such as climate. The colonies in each habitat-class are widely scattered in the region investigated, and their similarities are correlated only with the background, not at all with proximity. Figure 2 is a map of the Oxford district showing all the localities where colonies have been found. These colonies are isolated from one another by unsuitable habitats. It is obvious that colonies from mixed deciduous woodland agree in composition, although often separated and surrounded by colonies in rough herbage or hedgerows, which themselves agree within their habitat class, not according to their relative geographical positions. Geographical variation in the proportions of the shell-types tends to follow variation in the background very faithfully. At Rockley (SHEPPARD 1952) a single widespread colony occurs in downland and in three isolated beechwoods, separated by the downland. The different parts of the colony show a remarkable correlation with their backgrounds. A similar situation has been found at Tackley, north of Oxford, and Pusey Common Wood, ten miles west, where hedgerow colonies approach within a few feet of colonies in woodland without losing their distinctive characters (Pusey Common Wood and contiguous hedgerows on Oxford side and further side; Tackley Heath Wood, and Tackley Heath in tables 2, 3 and 4). That such situations can be found is the strongest possible evidence both for selection and for a very low degree of migration. LAMOTTE (1951) and SCHNETTER (1950) have found experimentally that migration is

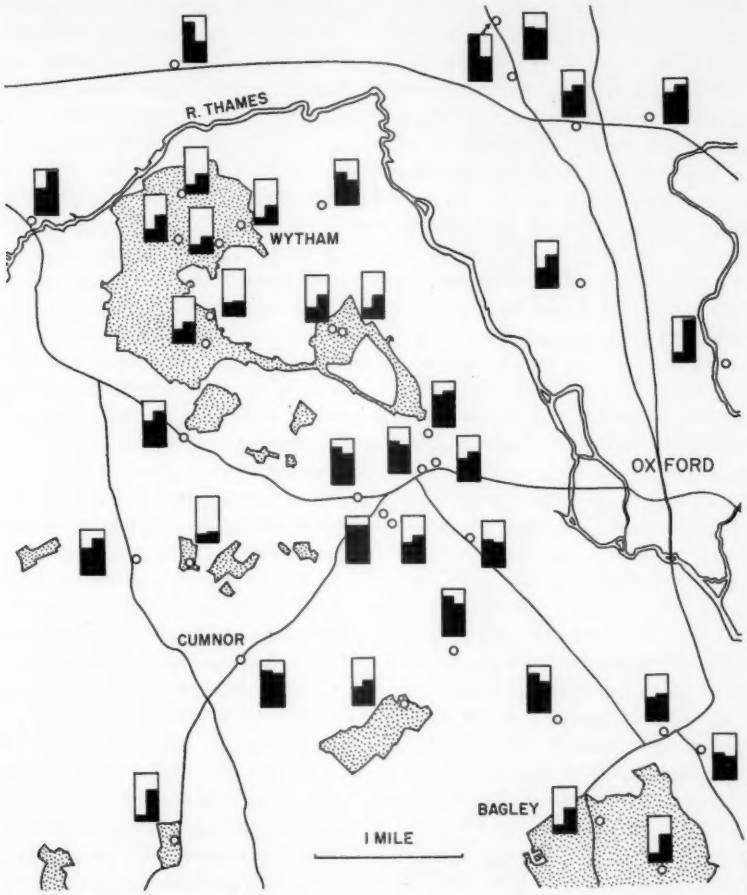


FIGURE 2.—Sketch-map of the Oxford district showing for each colony found % yellow shells (left hand column) and % effectively banded shells. Woodlands stippled; all colonies outside them are in hedgerows or rough herbage.

indeed very restricted. On the basis of his experiments, LAMOTTE concluded that no colony of more than 30 metres radius can be considered a panmictic unit, nor can linear colonies longer than 52.5 m. Both LAMOTTE, and SCHNETTER who obtained a rather higher value for the panmictic unit, used marked snails placed within a colony, and noted that they dispersed rather rapidly at first, becoming more sedentary later on. It is possible that if snails living in the colony had been marked individually and each immediately replaced where it was found, the estimate for the panmictic unit might have been even smaller.

Selection acts within as well as between the main banding and colour classes.

It has been shown (CAIN and SHEPPARD 1952) that not only the proportion of pinks and browns in a colony but also the shade of the pink shells is determined by selection, rich reds being found on beech leaf litter for example, but pale pinks and fawns on oak litter, which is a much paler brown. Selection also appears to act on the character *vestigial bands,* or traces. These appear only as small dark marks close to the lip in the normal band-position in the adult shell (p. 90). A shell with five traces may be genetically 12345, but phenotypically it is 00000. DR. LAMOTTE has very kindly informed us that in scoring his samples he counted all traces as full bands. We have ignored them as phenotypically of little importance. We find that the use of his system makes no important difference in the proportions of unbanded shells in our colonies; however, the data suggest that the unbanded individuals with traces are more abundant in woodlands than in hedgerows and rough herbage. This is to be expected, since the proportions of unbanded shells are low in these last two habitat classes, and banding is at an advantage. Consequently there will be selection against any modifiers causing banded shells to appear even partially unbanded. In woodlands, on the other hand, where unbanding appears to be at an advantage, such modifiers will be favoured. We have also noticed that in woodland colonies uninterrupted bands are often rather pale and narrow, while in hedgerows, even those contiguous with woodlands, the banding is usually more emphatic. These variations in breadth and intensity are difficult to score quantitatively.

Selection acts differently upon different combinations of shell characters. As already shown, the effects will be appreciable by examining wild populations only when there is strong linkage. Linkage between colour and unbanding, 00000, is sufficiently strong (p. 91) for these effects to be observed, and in fact it can be seen from tables 2–6 that yellow unbanded shells tend to be deficient in woodlands ($P < 0.01$) and to be in excess in some other habitats. An interesting exception is at Great Copse, Chislehampton where yellow 00000 is in excess, but the background here is, for a woodland, excessively green (p. 100). This variation in frequency of yellow unbanded shells is exactly what would be expected, as a result of visual selection in relation to the background, since when yellow is conspicuous as on brown backgrounds the plain yellow unbanded shells will be more obvious than those partly obliterated by banding. BOETTGER (1931) has already stated that unbanded yellow shells are never found in dense woodlands, and are common in open sunny places, but unfortunately does not give the necessary data. Our results support his view, although we do not usually find a complete absence of yellow unbanded shells in woodlands.

Visual selection acts not only on the shell but also on the rest of the visible phenotype. The colour of the snail's body varies from nearly white through grey to black, almost independently of shell colour and banding. The mean shade of the body-colour in each colony is strongly correlated with both the percentage of yellows in the colony (itself correlated with the greenness of the background) and with the exact shade of the pink shells (CAIN and

SHEPPARD 1952). The darker bodies, as might be expected, predominate on the darker backgrounds, and selection acts upon body colour although the body of the snail is far less often seen than the shell.

Non-visual selection

BOETTGER (1931) states, without giving details, that the degree of diversity in a colony is proportional to the diversity of its background. All our results agree entirely with this statement. On smooth uniform backgrounds such as beechwoods and short turf, the colonies are far less variable than in hedgerows, rough herbage, or even mixed deciduous woodland. Full details for some of the colonies investigated are given by CAIN and SHEPPARD (1950).

On uniform backgrounds, visual selection reduces the frequency of most of the varieties, and would be expected to remove them if the background remains constant. SHEPPARD (1951) has found that in Reserve Ride (Wytham Woods) of 1358 marked snails released on April 26th, 1950, 46 predated shells were actually found at thrush stones in the short period between 28th April and 5th June 1950. This indicates that about 3% of the population was predated in this period. At the beginning of the period, when the background was brown, yellow shells were at a disadvantage. His figures for selective destruction of yellows at the beginning of the period for three independent groups of shells are consistent and suggest that on the thrush-stones about 1.5 times as many yellows appeared as would be expected if predation was random. If the background were to remain constant (as it does in beechwoods and in many grassy places) then even with no more selective predation per year than that found in Reserve Ride between 26th April and 5th June 1950, it is clear that there would be selection against yellow shells on a brown background (or for them on a green one) of the order of 1% or more. This would remove either the yellows or the other colours from the colony relatively rapidly. A tentative estimate of population size at Marley Bog made by recapturing marked snails, gives about 10,000 adults present. From the degree of predation shown in table 7 between 4th July 1951 and 20th July 1951, it is obvious that selection for unbanded shells at this locality, with a constant background would be of at least the same intensity.

BOETTGER (1950b) and DIVER (1929) have pointed out that *C. nemoralis* has been polymorphic for banding at least since Neolithic times, and probably much longer. All its close relatives are polymorphic for banding and colour. Polymorphism in *C. nemoralis* must therefore be regarded as normal and stable. But it might be maintained either by selection for different varieties in different places, combined with gene-flow, or by mechanisms tending to maintain variability independent of the locality, such as that described by FISHER (1930a). The simplest situation is found when in a single pair of allelomorphs the heterozygote is at a selective advantage to both homozygotes. All the colonies we have investigated have been polymorphic, and of more than 800 colonies investigated by LAMOTTE (1951) only two are apparently monomorphic (yellow unbanded). To maintain polymorphism in all these colonies, many of

which are well isolated geographically, migration must be frequent and widespread, unless visual selection is very low.

The remarkable correspondence found by us, of parts of colonies with their different backgrounds and the extensive experiments of LAMOTTE (1951) show that migration in *C. nemoralis* is very slight (except possibly by human aid in urban districts), and cannot maintain polymorphism in face of the high visual selection demonstrated by us. Therefore there must be some nonvisual selective pressure, of the type discussed by FISHER (1930a), which is stabilising the polymorphism.

There is good indirect evidence for nonvisual selection within the class of brown shells. We formerly considered (CAIN and SHEPPARD 1950) that there was some interference between brown and the expression of banding. Further experience in collecting shows that the faint fluctuations of ground colour simulating very weak banding in browns occur also in pinks and yellows, and that banding when it does occur in browns is as clear as in banded shells in the other colour classes in the same colony. As tables 2–6 show, banded browns are always far less common than unbanded ones. There is an excess over expectation of unbanded brown shells even if it be assumed that brown is an allelomorph of banding, not colour, and that the brown heterozygotes are unbanded and only the brown homozygotes are banded. This unlikely assumption much reduces the expected frequency for banded browns. Moreover, since the deficit of banded browns is independent of the background of each colony, and therefore of the direction of visual selection of banding, the assumption that brown is an allelomorph of banding leads to the conclusion that there is constant nonvisual selection against banded browns. If brown is not an allelomorph of banding but is closely linked to it, then again there must be selection constantly against banded browns irrespective of the background. No doubt visual selection will act on banded as against unbanded shells in brown as in the other colour classes. But since brown is by far the darkest of the general shell colours, and most like the bands in hue, it is to be expected that visual selective discrimination between banding classes in brown will be less than in any other colour-class. In fact, in this colour class only, it seems constantly unable to override the effects of nonvisual selection.

Direct evidence of nonvisual selection has not been recorded in *C. nemoralis*. SCHNETTER (1950) has shown very strong selection to be favouring yellow unbanded *C. hortensis* in a colony which he observed from 1942 to 1950. He finds that this variety is more abundant in drier and more open places, and correlates its increase with a drying out and clearing of the locality. However, thrushes feed in this colony, and it is possible that part of the increase may be due to visual selection. In *Fruticicola lantzi*, however, GAUSE and SMARAGDOVA (1940) have shown that there is a considerable difference between dextrals and sinistrals in their capacity to resist starvation.

MAINTENANCE OF THE VARIATION

Different authors have come to very different conclusions about the relative importance of selection, mutation, migration and genetic drift in maintaining

the extraordinary variability of Cepaea. BOETTGER (1931, 1950a) considers that the degree of variation is controlled by the diversity of the background through visual selection, and that on uniform backgrounds the number of varieties will be reduced unless replenished by natural or artificial introductions. As LAMOTTE (1951) points out, BOETTGER has not published the data necessary to support his conclusions. DIVER (1940) believes, on the contrary that variation in *C. nemoralis* is random, and that the difference between colonies is due to genetic drift, but his conclusions are equally unsupported by published evidence.

As just stated, SCHNETTER (1950) has found in *C. hortensis* evidence of strong selection which he correlates with a change in the environment. He did not investigate the relative importance of visual selection and climatic selection in detail but makes it clear that the nonvisual selection was the more important. He was unable to find evidence of similar selection in *C. nemoralis* living in the same area, but his investigations in the two species were not fully comparable since they differed very strongly in the proportion of varieties present. On evidence which, as he himself is careful to point out, is very inadequate, he believes that some geographical variation can be discerned in *C. hortensis,* not in *C. nemoralis,* and as widely separated colonies may be much more alike than nearly adjacent ones, he concludes that the overall influence of climatic selection must be very slight, and that a great deal of the variation between colonies is due to random extinction of genes (i.e., to genetic drift). This conclusion is entirely based on the assumption that the direction and intensity of selection cannot alter profoundly over short distances. Our results show that this assumption is incorrect (e.g., fig. 2).

SCHILDER (1950) has studied in considerable detail the colonies of *C. nemoralis* and *hortensis* on a small island off the north German coast. He mapped the distribution of each species and the occurrence of particular varieties, indicating for each collecting station the nature of the habitat (oak copse, cultivated land, etc.) and listing the principal plants present. He found that at certain stations, particular varieties were especially abundant, and that their frequencies tended to fall off rather uniformly with distance from these " centres." Both the ranges of the species and their geographical variation were quite different. Because he did not find these species in some apparently suitable localities, he considered that colonization of the island was still proceeding. Without giving a detailed analysis, he found no correspondence between habitat and the frequency of particular varieties. But he has not studied the appearance of the background in relation to local variation, and his floral data do not enable one to arrive at any estimate of it, since the same species of plant can produce very different backgrounds, depending on the density of individuals and their size. SCHILDER interprets these results as indicating that variation in these species is independent of the environment. He believes that it is maintained by introduction or mutation, the new forms spreading out from their place of origin in the colony by slow gene-flow. It is quite possible that colonization of this island is recent and still in progress, and that importations from outside are continuing. In this case, the colony is not in equilibrium with its

environment, and one can draw no conclusions from an apparent absence of selection. But it should be noted that the variation-pattern observed by SCHILDER could be brought about in two ways. There could be either the introduction or local production of new forms in already colonized areas, or the introduction of one or a few individuals into unoccupied territories. In the first case, the new form cannot reach a high frequency at the place of origin in the face of continual gene-flow without a considerable selective advantage, yet that gene-flow is high seems demonstrated by the length and smoothness of the character-gradients claimed by SCHILDER. In the second case, local homogeneous populations will be produced. If these reach high population densities, and expand, they will eventually come into contact with other populations, interbreed, and produce clines, which will gradually disappear. Neither of these hypotheses is incompatible with the control of genes in the colonies primarily by selection.

In fact, gene-flow in these colonies is only inferred not demonstrated, since demonstration requires observations made at two separate periods, at least. But it is remarkable that all the unbanded *nemoralis* are pink, although yellows are present in other banding-classes. This can only mean that either there is strong selection (with linkage) against unbanded yellows, or that the unbanded allelomorph has not been on the island long enough for an appreciable frequency of the crossover class to appear. Even if there were absolute linkage (which is unlikely) one would still expect unbanded yellows to appear either by further introduction or by mutation, especially if LAMOTTE's estimate for this mutation rate in Cepaea can be believed (p. 107).

By far the most important single paper on Cepaea that has yet appeared is LAMOTTE's study of *C. nemoralis* (1951). This author's work on population-size, panmictic units, homogamy, and migration is the best yet produced. He is also the first to apply quantitatively SEWALL WRIGHT's equations for the interaction of selection, mutation, migration and population size to an ensemble of actual populations. His conclusions, based on a careful study of over 800 French colonies, are diametrically opposed to ours, since he believes that mutation assisted by migration is the prime agent maintaining the variation, that differences between colonies are due to genetic drift, and that selection, although present, plays an almost negligible part. In the colonies investigated by us, selection is the primary factor both in maintaining variation within a colony and in determining the differences between colonies. This apparent contradiction must be investigated.

LAMOTTE regards selection as unimportant for the following reasons.

(1) He finds no obvious correlation between variation in colonies and the environment.

(2) Although there is some selective predation by thrushes, these birds are only winter-visitors in France, and in winter most of their predation is non-selective.

(3) He can find no correlation whatever between variation in *C. nemoralis* and *C. hortensis* living together in the same place, on the same background.

(4) His data show that small colonies are more variable than larger ones.

(5) He has been able to fit to his data on the frequency of the gene for bandless in Aquitaine a curve derived from SEWALL WRIGHT'S formulae, which indicates that in these colonies migration plus mutation is important and selection almost negligible. Using additional data from other areas he concludes that the mutation rate of the allelomorph for banding to bandless is 1×10^{-4}, and the reverse rate 5×10^{-4}, and that these mutation rates are sufficient to maintain polymorphism. He supposes therefore, that selection never plays more than an accessory part (1951, p. 230).

These reasons, and the conclusions based upon them, seem incorrect, on the following grounds respectively.

(1) Many of the localities investigated by LAMOTTE were, as he points out, in recently disturbed or unstable environments, and in these a correspondence of varietal proportions and the background is not to be expected. Also in many localities there was a mixture of habitats, such as we have avoided (p. 98, above) so that even if the proportions of phenotypes were controlled by visual selection, a correspondence could hardly be observed.

LAMOTTE makes no direct comparison of colonies with backgrounds, for reasons considered below. He does make comparisons of colonies classified by their broad habitats (1951, p. 135), but using such classes as " bottoms of valleys," " dry biotopes " and " sunny places " which are too broad to be useful in considering backgrounds or investigating nonvisual selection if visual selection is effective. However, he does use two classes, oak coppices, and hedges, which seem to be sufficiently homogeneous, and comparable to our classes, although as he gives no description of them it is not certain that several types may not be included. He considers only the proportion of unbanded to banded shells: a better estimate involves the proportions of effectively unbanded shells (p. 98, above) and of the various colour classes. His data indicate that variation between colonies in hedgerows is greater than in oak coppices, as might be expected. The mean frequency of unbanded shells, however, is higher in both classes than in our data, and is not significantly lower in hedgerows than in oak coppices. Since the backgrounds have not been described, no definite conclusion can be reached. It is quite possible that the observed higher frequencies of unbanded shells in his colonies could be due to climatic selection. SCHNETTER (1950) in *C. hortensis* finds that, largely as a result of nonvisual selection, unbanded shells are more common in open, dry, sunny places. LAMOTTE (p. 136) obtained indications of the same situation in *C. nemoralis*. Moreover, his data on climatic factors suggest that the highest frequency of unbanded shells occurs in regions where the July temperature is highest. As insolation is considerably higher in France than in Great Britain, a higher proportion of unbanded shells might be expected in France especially in the more open habitats such as hedgerows in contrast to woods.

LAMOTTE finds no evidence for broad climatic selection. However, to investigate this, he has combined all colonies in groups according to the values of particular climatic factors. If local selection of the type we have demon-

strated is acting, then grouping the colonies by climatic characters alone will mask more or less completely the effects of climatic selection. Nevertheless his published results (p. 141) do suggest a correlation between the frequency of unbanded shells and temperatures in both July and January, but these two factors may not be independent. It is possible that if the effect of visual selection had been eliminated by considering only colonies on the same background, a significant correlation with climate could have been observed.

(2) In Britain the song thrush is certainly not the only bird that preys upon these snails (WITHERBY et al. 1943), and in Europe many birds are recorded as eating molluscs (KLEINER 1936, 1937). Half-grown individuals, in which colour and banding are well developed, could be eaten whole by many small birds, including, of course, thrushes, which need to bring only the larger individuals to the anvils. It is extremely unlikely that a scarcity of song thrushes in any district means the virtual absence of visual predators.

In about 40 of his colonies, LAMOTTE was able to obtain sufficiently large samples of shells broken by predators to make a comparison between them and intact shells. His illustration (p. 183) of shells broken by birds includes at least two which have every appearance of having been attacked not by birds but by mammals. (This conclusion has been very kindly confirmed for us by MR. D. J. MORRIS, who has made a special study of the subject, MORRIS, in press.) It is most unlikely that rodents and birds find the snails in the same way, so that combining all the predated shells in a colony may well obscure the effects of selection. Nevertheless, LAMOTTE has obtained evidence of visual selection in certain colonies, always against unbanded shells; in others he found no evidence of selection, although the snails were being attacked, and concluded that in these there was only nonselective winter predation by thrushes. He has not, however, excluded the possibility of important visual selection by other birds. He compared the proportions of unbanded shells in colonies with the presence or absence of visual selection, and found no correlation. But because selection can vary in intensity and direction, the average selective value of unbanded shells should be compared with their frequency in each colony. Only those colonies that have lived in the same habitat for a long period should be used, and to reduce the effects of nonvisual selection, comparison should be made only between those in the same sort of habitat and in the same area.

LAMOTTE has compared the diversity in frequency of unbanded shells in colonies known to be attacked by predators and in those he supposes to be exempt, and finds no difference. He comments on the high degree of diversity in both classes. But in fact, it is extremely likely that all these colonies are attacked by predators (many of which do not leave broken shells as evidence), and the high diversity is to be expected if visual selection is operative against a complicated mosaic of backgrounds, favouring unbanded shells on some and banded on others.

(3) Apart from all other considerations, LAMOTTE (1949, 1951) believes that it is possible to demonstrate conclusively that there is no selective in-

fluence of the environment upon the visible characters of the snail. The two very similar species *C. nemoralis* and *C. hortensis* are not infrequently found living together in the same colony. If visual selection is favouring a particular variety in one species in a mixed colony then it should favour it in the other species as well. Consequently there should be a correlation between the frequency of unbanded shells in *C. nemoralis* and *C. hortensis* in mixed colonies. LAMOTTE finds no trace of such a correlation (and neither do we).

But in fact such a correlation is not to be expected; and the stronger the visual selection the less likely it is to be found. With visual selection acting on phenotypes, the same banding patterns displayed on one ground-colour of the shell will not have the same selective values when on other shell-colours. In woodlands, where unbanded pink shells are at an advantage, there is nevertheless an excess of banding in the yellow as compared with the pink class in *C. nemoralis,* since the banding obscures the disadvantageous colour (p. 102, above). As yellow is far more common in *C. hortensis* than in *C. nemoralis,* one would expect to find—and one does find—that in *C. nemoralis,* unbanded pink shells are common in woodlands, but in *C. hortensis* banded yellows. On smooth green herbage, on the other hand, from the point of view of visual selection, a high proportion of unbanded shells would be expected in both species, so that there will not be a simple negative correlation.

TABLE 8

Locality	*C. nemoralis*						*C. hortensis*					
	Yellow		Pink		Brown		Yellow		Pink		Brown	
	Unbanded	Banded	Unbanded	Banded	Unbanded	Banded	Unbanded	Banded	Unbanded	Banded	Unbanded	Banded
Rockley 1, 2 & 3	37	33	77	12	74	4	1	35	0	0	0	0
Rockley 9	1	3	2	7	9	3	2	8	0	0	1	0
Ashbury	0	3	5	6	17	0	1	21	0	0	0	0
Beechgrove Farm Wood	0	5	31	16	7	0	2	13	0	0	0	0
Aston Rowant	0	0	23	7	0	0	0	3	0	0	8	0
Boarstall	0	0	54	79	0	0	2	16	0	0	16	1

Table 8 gives the proportions of banded and unbanded shells in both species in four mixed colonies investigated by us. Although there is a very striking correspondence between the background and the *C. nemoralis* population (see CAIN and SHEPPARD 1952; SHEPPARD 1952; and tables 2–6 in the present paper) there is little resemblance between the proportion of banded shells in the two species.

Furthermore, for a correlation to be apparent, the nonvisual selective values of the various allelomorphs (which SCHNETTER 1950 for unbanded *C. hortensis* found to be high and very susceptible to changes in habitat) must be nearly the same in both species, which is unlikely. Even if this were so, the

correlation might be very feeble unless the two species were intermixed either on a uniform background, or at uniform density in each distinct area of an irregular background, and were predated in the same way and to approximately the same degree. SCHILDER (1950) finds that *C. hortensis* is much more addicted to sitting on stems and in bushes, while *C. nemoralis* is more usually found on the ground. This is in agreement with our own observations. DR. P. H. T. HARTLEY informs us that in his experience thrushes cannot effectively predate snails resting more than a few inches above the ground. Consequently even in the same habitat, the incidence of predation by thrushes on the two species is likely to be different.

(4) Recently LAMOTTE (1952) has claimed that the differences between small colonies of *C. nemoralis* are considerably greater than between large ones in respect of the frequency of the genes for pink, and especially for absence of bands. He considers that since the effects of selection are independent of population size, any difference in variance between large and small colonies must be due to random fluctuations (genetic drift). According to his data, within a limited region, drift is responsible for one half the variance between large colonies (3,000–10,000 individuals) and five sixths that of small ones (500–1000). This conclusion is unacceptable for two reasons.

(a) Because backgrounds vary considerably over small distances, and the larger colonies will tend to be spread over larger areas and therefore over more types of background than the smaller ones, it is to be expected that large colonies will tend to be more alike, since the diverse effects of selection in different parts of each colony will tend to cancel out when the colony is considered as a whole, and in any case will be reduced to a certain extent by gene flow. Small colonies will tend to be on more homogeneous backgrounds, and will therefore become more diverse by selection. If selection is acting in different directions in different habitats, as we have shown, then when gene frequencies for all the colonies (in many different habitats) within a region are combined and compared with those of other regions, there should be less variance between frequencies for regions than between individual colonies in the same region. LAMOTTE's figures show that this is so, and therefore fail to provide any distinction between the effects of diverse selection and of genetic drift.

(b) It is far from certain that the effects of visual selection are independent of population size. Thrushes at least (HARTLEY unpublished) and probably many other birds, take these snails especially at periods when other food is scarce, or when an unusually large supply of food is necessary (nesting period), and they appear to be able to find as many individuals as are required for food at any one time. There is no evidence whatever that they experience much difficulty in obtaining the snails. Consequently there is no reason to believe that the intensity of predation is the same in small and large populations. If each thrush tends to take as many snails as it requires, irrespective of the size of the population predated, then the intensity of predation and the effects of visual selection will be greater in the smaller populations.

If polymorphism in *C. nemoralis* is balanced, then the gene-frequencies will depart further from those obtained solely by the action of nonvisual selection in the smaller colonies. One cannot assume without proof that visual selection is independent of population size, especially when birds that hold territories are involved.

It is clear that before differences in variance between large and small colonies can be advanced as a proof of the importance of genetic drift, one must compare only colonies each of which lives on a single type of background, and show that the intensity of predation in them is irrespective of size.

(5) In view of his demonstration that selection is unimportant, LAMOTTE considers that the agreement between his data on population-size, migration and the frequency of the allelomorph for bandless, on the one hand, and a curve derived from SEWALL WRIGHT's equations on the other, means that in this case the equations present an adequate analysis of the actual situation. Consequently they can be used to determine values for selection and the mutation rates, and to show how polymorphism is maintained. But this is so only

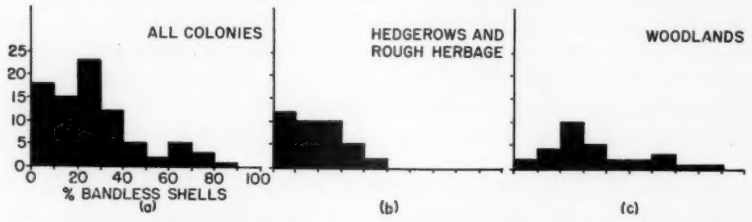


FIGURE 3.—Frequency histograms for % totally unbanded shells (as in LAMOTTE's diagrams) in colonies with samples of 50 or more. (a) All colonies; (b) hedgerows and rough herbage only; (c) woodlands only.

if selection is very feeble, and as we have shown above, his conclusions on this point cannot be sustained. But if selection is important, then it is quite possible that the characteristics of the various backgrounds might be such that exactly the same curve could be produced by strong visual selection. In our colonies the effect of visual selection is obvious. Yet when a histogram for the frequency of unbanded shells is plotted (fig. 3a) it shows a remarkable resemblance to that obtained by LAMOTTE for the regions he investigated in the Pyrenees (1951, p. 131). However, when values for colonies in rough herbage and in hedgerows, on the one hand, and for all woodland colonies on the other are plotted separately, two obviously different curves are obtained (fig. 3b, c), the first of which is almost exactly like Lamotte's frequency histogram for the Parisian region (1951, p. 130) and the second resembles that for Aquitaine.

It is clear from these results that to combine the data from a particular region without regard to background is to obscure the effects of visual selection. It should also be noted that, as LAMOTTE has carefully pointed out, to construct the necessary curve for comparison one must assume both that selec-

tion is approximately constant throughout the region considered (1951, p. 202) and that the selection coefficients are very small, of the order of size of mutation rates (1951, pp. 212, 213).

LAMOTTE's results lead him to postulate mutation rates of the order of 10^{-4} to maintain variation in this species. These values are far higher than those normally obtained. But since his data are in no way incompatible with ours, it seems far more likely that in France as in England, selection is the primary agent maintaining the polymorphism and controlling the differences between colonies.

The maintenance of polymorphism by selection can be brought about by two principal methods (FISHER 1930a). Either the genotypes concerned become advantageous when rare and disadvantageous when common, or certain gene-combinations stand in particular ratios of selective advantage to others. In the simplest case, the heterozygote of a single pair of allelomorphs or for a chromosomal rearrangement is at an advantage over both homozygotes.

Although there is no evidence for the first method in Cepaea, it remains a possibility, in view of the work of DE RUITER (1952). This author has investigated certain birds preying on very protectively coloured insects, and has shown that when they encounter one of these they search for others of the same sort for some little time. If one can conclude from this that the finding of one form decreases for a time the chances of finding another, then it is possible that in such polymorphic species as *C. nemoralis*, the most abundant varieties will be relatively more heavily predated. The birds are most likely in searching for food to notice the more conspicuous and the commonest varieties. If the more conspicuous varieties are rare, then after a time, the search for further individuals of these sorts being unsuccessful, the birds will stop looking for them. The chances are that they will then be started on another hunt by encountering one of the more abundant varieties, and they will be able to hunt successfully for this phenotype for a much longer time. This will decrease the chances of their finding more of the rare conspicuous forms. There is no evidence for this in Cepaea, and, in our experience, one does not find on thrush stones an excess of one common variety on one day and of a different one on another day. Although the possibility is remote, it does indicate how slight differences in the methods of predation used by different predators may produce very different selective effects.

The great importance in *C. nemoralis* of the second method, namely selection favouring certain gene combinations, has already been fully demonstrated above (p. 104). All the available evidence points to its being the principal mechanism for maintaining polymorphism in this snail. Nonvisual selection of certain character-combinations involving more than one locus has already been demonstrated between brown and banding, as has visual selection involving other colours and banding (pp. 102, 104 above). Moreover, polymorphism may be considerable in banding even when only one colour is present. This fact indicates that its maintenance is due in part at least to the physiological advantage of heterozygotes at each locus.

It is remarkable (FISHER 1930b; HALDANE 1930) that the combination of (i) considerable and stable polymorphism, (ii) close linkage between many of the loci concerned, and (iii) rather frequent occurrence of a genotype recessive to most of the others involved in the polymorphism, is common to widely different groups of animals, and has nearly always been found when species highly polymorphic for visible characters have been investigated genetically. It occurs, for example, in *Cepaea nemoralis* and *C. hortensis* (Gastropoda), in Lebistes (Teleostei), and in several genera of Orthoptera (Apotettix, Paratettix, Chorthippus). In *C. nemoralis* we have shown that selection coefficients certainly greater than 1% are acting. FISHER (1930b, 1939) has remarked on the probability of high selective values in Lebistes, and in Paratettix and Apotettix has demonstrated values ranging from 6% to at least 40%.

FISHER (1930b) has given reasons for believing that the dominance relationships are a necessary consequence of the close linkage. FISHER and HALDANE agree that duplications and translocations may be the causes of close linkage. Translocations, however, tend to be disadvantageous and rare in nature. Duplications have been inferred in Orthoptera (WHITE 1945). In recent years it has been found that inversions are relatively abundant in wild populations (WHITE 1951; DOBZHANSKY 1951), that they are associated with stable polymorphism in Drosophila, at least (DOBZHANSKY 1951), and that they have very high selection coefficients. The very different linkage values for colour and banding in *C. nemoralis* reported by FISHER and DIVER (1934) strongly support the view that chromosomal rearrangements, probably inversions, are present in *C. nemoralis*.

EVOLUTIONARY SIGNIFICANCE OF THE VARIATION

The evolutionary significance of polymorphism in Cepaea and other forms is highly obscure. There is no evidence whatever that if every population of *C. nemoralis* were to become monomorphic overnight, the species would not continue to survive in the same places and at the same densities as before. Its local geographical distribution is probably determined primarily by the nature of the soil, and the sizes of its populations by this and density-dependent factors. Any slight increase in predation that might occur as a result of a change to monomorphism could easily be compensated by a change in the balance due to the density-dependent factors.

SCHNETTER (1950) has suggested that polymorphism in *C. nemoralis* is adaptive. As the different varieties have different selective values, a population might be enabled to persist through large climatic variations by changing the proportions of the different varieties and thereby maintaining its density of individuals. DOBZHANSKY (e.g., 1951) has suggested that balanced polymorphism in general is adaptive, both in promoting persistence of the species and in enabling a larger variety of niches to be exploited. But this hypothesis rests on the unlikely assumption that because one of two varieties is inferior to the other when in competition with it in the same population, it is normally incapable under the same environmental conditions of persisting as a pure

population. Because yellow shells are at a disadvantage to others in beechwoods, it does not in the least follow that an entirely yellow population could not persist in a beechwood (in the absence of pinks). The relationships between the ecological factors that control the size of a population and the selective values of particular genotypes within it have never been adequately explored.

It would be very interesting to compare the density under different environmental conditions of breeding communities of Drosophila (in population cages) some of which are monomorphic for certain inversions and others polymorphic. DOBZHANSKY has suggested (1951, p. 123) that a population of *D. pseudoobscura* homozygous for the inversion ST is at a disadvantage under winter conditions. Exactly what is meant by a population being at a disadvantage is not certain. Presumably the population size is expected to decrease to a dangerously low level. This could be tested by the experiment suggested above. It will be rather surprising if any great difference in density between monomorphic and polymorphic populations is demonstrated.

As FISHER (1930a) has shown, whenever heterozygotes are at an advantage to both homozygotes, irrespective of the reasons for this advantage, stable polymorphism will result and persist, while if either homozygote has the highest advantage, only transient polymorphism will result. Consequently instances of stable polymorphism will accumulate, and will be frequently observed. Its widespread occurrence is evidence that it is maintained, once it has appeared, by selection, but not that it is, as such, of any *adaptive* value. Adaptation and selection are not synonymous, as has been clearly shown by FISHER (1930a), and SOMMERHOF (1950), and by CROSBY (1949) in the particular case of *Primula veris*.

SUMMARY

(1) The very variable European land snail *Cepaea nemoralis* has shown stable polymorphism since before the Neolithic period. The proportions of different varieties vary considerably from one colony to the next. It has been claimed that this situation is due to genetic drift.

(2) We have shown that this snail is subject to strong visual selection by birds, which results in a correlation between the varietal composition of each colony and the exact background on which it lives. This selection must be balanced by strong nonvisual selection, for the polymorphism to be maintained in each colony.

(3) The genetics of the variation is remarkably similar to that in other unrelated polymorphic species in which also high selective values have been demonstrated or inferred. Most of the loci investigated (at least 3 and probably 4) are linked, and one very frequent variety is recessive to all the other common ones (universal recessive). The degree of linkage varies, and it is probable that the loci concerned are associated with inversions.

(4) LAMOTTE (1951) after a very careful study of French colonies, has concluded that polymorphism is maintained by high rates of mutation (greater

than 10^{-4}) and that selection is negligible, also that the differences between colonies are due to genetic drift. These conclusions are shown to be unacceptable, since they are based on data grouped in such a way as to obscure the effects of visual selection, and depend on theoretical considerations involving the assumption that selection is negligible, which is invalid.

(5) Although polymorphism in this and other species is controlled by selection, there is no evidence that it is adaptive and contributes to the persistence or ecological expansion of the species. Theories of adaptive polymorphism rely on an unlikely assumption concerning the relations between factors controlling the density and persistence of populations on the one hand, and the relative selective values of alternative genotypes within them, on the other.

ACKNOWLEDGEMENTS

We are deeply indebted to DR. M. R. SAMPFORD for the statistical analysis, and to DR. E. B. FORD who read this paper in manuscript. Grateful acknowledgement is made to the Nuffield Foundation for its generous support to one of us (P. M. SHEPPARD).

LITERATURE CITED

BOETTGER, C. R., 1931 Die Entstehung von Populationen mit bestimmte Variantenzahl bei der Landschneckengattung *Cepaea* Held. Zeit. ind. Abst. Ver. **58:** 295–316.

1950a Analyse einer bemerkenswerten Population der Schnirkelschnecke *Cepaea hortensis* Müller. Abhand. der Braunschweig. Wiss. Gesell. **2:** 1–12.

1950b Die seit dem Pleistozän erfolgte Änderung in der geographischen Verbreitung je einer bestimmten Mutante der Landschneckenarten *Cepaea nemoralis* L. und *Bradybaena fruticum* Müll. Syllegomena biologica 56–67.

CAIN, A. J., 1951a So-called non-adaptive or neutral characters in evolution. Nature **168:** 424.

1951b Non-adaptive or neutral characters in evolution. Nature **168:** 1049.

CAIN, A. J., and P. M. SHEPPARD, 1950 Selection in the polymorphic land snail *Cepaea nemoralis*. Heredity **4:** 275–294.

1952 The effects of natural selection on body colour in the land snail *Cepaea nemoralis*. Heredity **6:** 217–231.

CARTER, G. S., 1951 Animal Evolution. A Study of Recent Views of Its Causes. London: Sidgwick and Jackson.

CROSBY, J. L., 1949 Selection of an unfavourable gene-complex. Evolution **3:** 212–230.

DIVER, C., 1929 Fossil records of Mendelian mutants. Nature **124:** 183.

1932 Mollusc genetics. Proc. VI. Int. Congr. Genetics (Ithaca) **2:** 236.

1940 The problem of closely related species living in the same area. In The New Systematics (ed. J. S. Huxley). London: O.U.P.

DOBZHANSKY, T., 1941 Genetics and the Origin of Species. 2nd ed. New York: Columbia.

1951 Genetics and the Origin of Species. 3rd ed. New York: Columbia.

FISHER, R. A., 1930a The Genetical Theory of Natural Selection. London: O.U.P.

1930b The evolution of dominance in certain polymorphic species. Amer. Nat. **64:** 385–406.

1939 Selective forces in wild populations of *Paratettix texanus*. Ann. Eug. **9:** 109–123.

FISHER, R. A., and C. DIVER, 1934 Crossing-over in the land snail *Cepaea nemoralis* L. Nature **133:** 834.

FORD, E. B., 1940 Polymorphism and taxonomy. In The New Systematics (ed. J. S. Huxley) London: O.U.P.

GAUSE, G. F., and N. P. SMARAGDOVA, 1940 The decrease in weight and mortality in dextral and sinistral individuals of the snail *Fruticicola lantzi*. Amer. Nat. **74**: 568–572.

HALDANE, J. B. S., 1930 A note on Fisher's theory of the origin of dominance, and on a correlation between dominance and linkage. Amer. Nat. **64**: 87–90.

1932 The Causes of Evolution. London: Longmans, Green.

HUXLEY, J. S., 1942 Evolution, the Modern Synthesis. London: Allen and Unwin.

KLEINER, A., 1936 La consommation des mollusques par les oiseaux. L'Oiseau et la Revue française d'Ornith. **2**: 233–251.

1937 Über die Conchylien Aufnahme der Vögel. C. R. XII Congr. int. Zool. Lisbonne 1935. 1805–1824.

KLEINER, E., 1912 Untersuchungen am Genitalapparat von *H. nemoralis, hortensis* und einer weiteren Reihe von Lang gezüchteter Bastarde der beiden Arten. Zeit. ind. Abst. Ver. **9**: 216–262.

LAMOTTE, M., 1949 Sur le role sélectif de l'aspect phénotypique des variétés de l'escargot des bois (*Cepaea nemoralis*) C. R. Acad. Sci. **228**: 1353–1354.

1951 Recherches sur la structure génétique des populations naturelles de *Cepaea nemoralis* (L.). Bull. Biol. France Suppl. **35**: 1–239.

1952 Le rôle des fluctuations fortuites dans la diversité des populations naturelles de *Cepaea nemoralis* (L.). Heredity **6**: 333–343.

LANG, A., 1904 Ueber Vorversuche zu Untersuchungen über die Varietätsbildung von *Helix hortensis* Müll. und *Helix nemoralis* L. Denkschr. d. Med. Naturwiss. Gesellsch. Jena **11**: 439.

1908 Ueber die Bastarde von *Helix hortensis* Müller und *Helix nemoralis* L. Festschr. Univ. Jena.

1912 Vererbungswissenschaftliche Miszellen. Zeit. f. Ind. Abst. Ver. **8**: 233–283.

MAYR, E. 1942 Systematics and the Origin of Species. New York: Columbia.

PERROT, J. L., and M. PERROT, 1938 Monographie des Helix du groupe *Cepaea*; contribution à la notion d'espèce. Bull. Biol. **72**: 232–262.

ROBSON, G. C., and O. W. RICHARDS, 1936 The Variation of Animals in Nature. London: Longmans.

DE RUITER, L., 1952 Some experiments on the camouflage of stick caterpillars. Behaviour **4**: 222–232.

SCHILDER, F. A., 1926 Zur Variabilität von *Cepaea*. Zeit. ind. Abst. Ver. **39**: 249–280.

1950 Die Ursachen der Variabilität bei *Cepaea*. Biol. Zbl. **69**: 79–103.

SCHNETTER, M., 1950 Veränderungen der genetischen Konstitution in natürlichen Populationen der polymorphen Bänderschnecken. Verh. deut. Zool. Marburg. **1950**: 192–205.

SHEPPARD, P. M., 1951 Fluctuations in the selective value of certain phenotypes in the polymorphic land snail *Cepaea nemoralis* (L.). Heredity **5**: 125–134.

1952 Natural selection in two colonies of the polymorphic land snail *Cepaea nemoralis*. Heredity **6**: 233–238.

SOMMERHOF, G., 1950 Analytical Biology, Oxford.

TAYLOR, J. W., 1914 Monograph of the Land and Freshwater Mollusca of the British Isles **3**. Leeds (England): Taylor.

WHITE, M. J. D., 1945 Animal Cytology and Evolution. London: Cambridge University Press.

1951 Structural heterozygosity in natural populations of the grasshopper, *Trimerotropis sparsa*. Evolution **5**: 376–394.

WITHERBY, H. F., F. C. R. JOURDAIN, N. F. TICEHURST and B. W. TUCKER, 1943 The Handbook of British Birds. London: Witherby.

CHROMOSOME BEHAVIOR IN FERTILE TRIPLOID ASTER HYBRIDS[1]

CHARLOTTE J. AVERS
Department of Botany, Indiana University, Bloomington, Indiana[2]

Received June 8, 1953

THE sterility of triploids and other unbalanced polyploids is generally conceded to be a consequence of irregularities in the distribution of univalents and multivalents at meiosis. Occasional viable gametes are produced, however, because of nonrandom orientation and segregation of chromosome associations. Thus, SATINA and BLAKESLEE (1937a, b) found a higher frequency of n and 2n chromosome numbers in gametes produced by triploid Datura than was expected on the basis of random orientation of trivalents.

The Canina roses furnish a striking exception to the general rule of sterility of unbalanced polyploids (FAGERLIND 1940). There are also several reports of fertile triploid and aneuploid individuals in other species. DEMOL (1921) found that triploid *Hyacinthus orientalis* L. (3n = 24) was fertile, as were various aneuploid strains in this cultivated species. Microspore chromosome numbers of 8 to 14 were found (BELLING 1924) and the fertile progeny contained somatic chromosome numbers of 16 to 30. A triploid form of *Crepis artificialis* (3n = 36) was obtained in which 17 bivalents and 2 univalents regularly formed (COLLINS et al. 1929). This behavior resulted in 62 to 70 percent pollen fertility in the triploids.

In the course of a biosystematic study of several species of Aster (AVERS 1953), three fertile triploid F_1 hybrids were obtained. Their unusual chromosome behavior has furnished the basis for the present report.

MATERIALS AND METHODS

Twenty separate crosses between *Aster shortii* Lindl. ssp. *shortii* (n = 18) and *A. cordifolius* L. (n = 9) were attempted in 1950, and three of these yielded one F_1 hybrid each. One of these three hybrids was successfully backcrossed to the tetraploid parent species in 1951, and nine backcross plants were raised to maturity. In addition to the experimentally obtained individuals, one natural triploid also has been studied cytologically. All plants were grown in the greenhouse at Indiana University.

Buds of the 13 plants studied were fixed in chloroform-ethanol-acetic acid, 4:3:1, for one week, and squash preparations of pollen mother-cells were made in aceto-orcein. Pollen fertility was determined by staining with cotton blue.

[1] Part of a thesis submitted to the Faculty of the Graduate School of Indiana University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Present address: Botany Department, Connecticut College, New London, Connecticut.

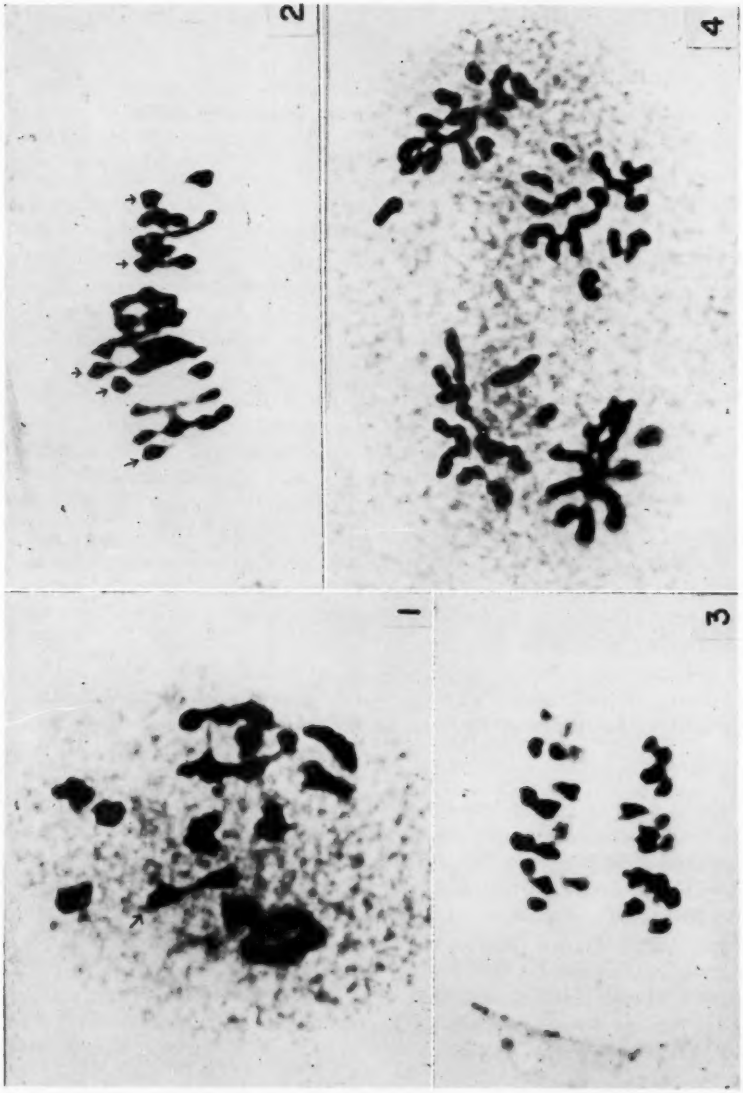


PLATE I.—Meiosis in fertile triploid Asters ($3n = 27$). × 2000.

TABLE 1

Chromosome constitution of three triploid F_1 Aster hybrids (3n = 27).

No. cells	Configurations			Expected gametic chromosome numbers[a]	
	Trivalents	Bivalents	Univalents	1 division	2 divisions of univalents
1	4	7	1	11–16	12–16
1	4	5	5	9–18	14–18
2	3	8	2	11–16	13–16
1	3	7	4	10–17	14–17
1	3	6	6	9–18	15–18
2	2	10	1	12–15	13–15
3	2	9	3	11–16	14–16
11	2	8	5	10–17	15–17
7	2	7	7	9–18	16–18
1	2	6	9	8–19	17–19
1	1	11	2	12–15	14–15
10	1	10	4	11–16	15–16
19	1	9	6	10–17	16–17
11	1	8	8	9–18	17–18
4	0	12	3	12–15	15
10	0	11	5	11–16	16
8	0	10	7	10–17	17
7	0	9	9	9–18	18
100	1.7	8.5	4.8	13–14	16

[a]Chromosome numbers are based on the following assumptions: (1) random distribution of univalents; (2) random orientation of trivalents; and (3) regular disjunction of all trivalents 2-by-1. The ranges of gamete chromosome numbers were determined empirically.

F_1 hybrids. All three hybrids were 90 percent pollen-fertile and contained 27 chromosomes in varying configurations at meiosis (table 1). In many microsporocytes it was seen that some univalents had begun to divide at diakinesis (fig. 1), and that all univalents divided at metaphase I (fig. 2). Although the univalent halves appear to disjoin precociously, their actual separation occurs synchronously with bivalent and trivalent segregation (figs. 3 and 5). Infrequently first anaphase cells were seen in which a lagging univalent was in the process of division. The nuclei enter a protracted interphase and at metaphase II the chromosome number exceeds 27. Sister nuclei contain 14 to 18 chromosomes, thus confirming the direct observations of MI

FIGURE 1.—Diakinesis showing 2 trivalents, 8 bivalents and 5 univalents. Arrow indicates precocious separation of univalent halves.

FIGURE 2.—Metaphase I showing dividing univalents.

FIGURE 3.—Anaphase I with 14 chromosomes going to one pole and 15 to the other, showing simultaneous separation of all chromosome associations.

FIGURE 4.—Anaphase II showing 16 chromosomes in each of two sister nuclei and 14 chromosomes in another nucleus of the tetrad. The fourth nucleus, with 14 chromosomes, is obscured. This indicates that the univalents have completed two successive divisions since the combined chromosome number exceeds 54, which would have been expected if the univalents had divided only once.

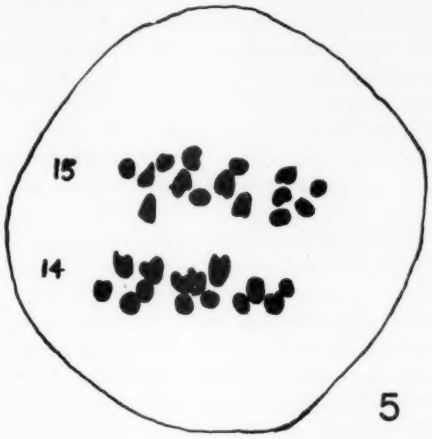
TABLE 2

Comparison of expected and observed gametic chromosome numbers in three F_1 triploids.[a]

Chromosome number	12	13	14	15	16	17	18	19
Expected[b]								
No. cells (103)	0.06 ± 3.1	1.03 ± 1.0	3.67 ± 3.3	16.48 ± 4.1	34.96 ± 4.9	31.16 ± 4.1	15.39 ± 3.0	0.26 ± 3.1
Per cent cells	0.06	1.00	3.56	16.00	33.94	30.25	14.94	0.25
Observed[c]								
No. cells (103)	0	1	12	21	37	22	10	0
Per cent cells	0.0	1.0	11.6	20.4	35.9	21.4	9.7	0.0
Dev./S.E.	0.0	0.3	2.5[d]	1.1	0.4	2.2[d]	1.8	0.1

[a]Calculations are based on data presented in table 1.
[b]Determined by expansion of the binomial for each configuration listed in table 1.
[c]Counts were made of individual second anaphase nuclei.
[d]Indicates statistical significance.

univalent divisions. There is great difficulty in obtaining adequate preparations of anaphase II for purposes of counting potential gamete chromosome numbers since the meiotic products are arranged tetrahedrally. However, counts were made of 103 nuclei in 31 cells, and in every case the chromosome number fell



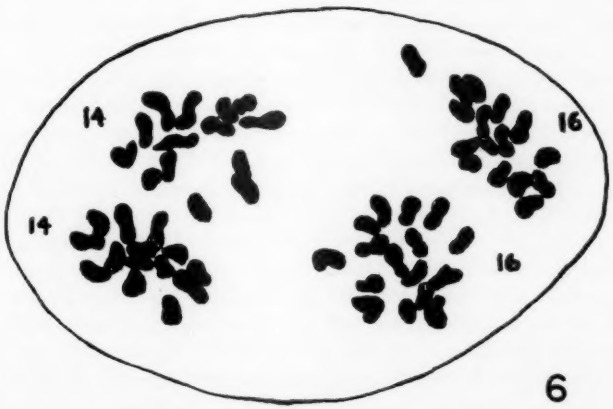


FIGURE 5.—Diagram of figure 3. × 2000.
FIGURE 6.—Diagram of figure 4. × 2000.

within the range expected on the basis of two divisions of the univalents (table 2). In most cases in which sister nuclei could be counted at anaphase II, it was seen that they contained the same chromosome number. Figure 4 is a photomicrograph of a second anaphase cell in which 16 chromosomes are visible in each of two sister nuclei and 14 chromosomes can be seen in a third

nucleus. The fourth nucleus in this cell also contains 14 chromosomes although this is obscured since they were in another focal plane (see fig. 6). No micronuclei were observed in any of the material. Rarely a chromosome was seen to be excluded from a telophase nucleus.

Backcross hybrids. Crosses of the triploid to the diploid parent species were unsuccessful. This behavior is typical of heteroploid crosses in Aster where it has been shown that the diploid × tetraploid cross usually fails (AVERS 1953).

Nine backcross hybrids were raised to maturity. Four of the backcross plants were obtained from the cross *A. shortii* (2n = 36) × F_1 *A. shortii* × *cordifolius* (3n = 27) and the remaining five plants were obtained from the reciprocal cross. The chromosome numbers of the backcross progeny ranged from 2n = 33 to 2n = 35. It is significant that the chromosome numbers of this random sample are within the range expected on the basis of two divisions of the univalents in the triploid parent. Phenotypically the backcross individuals are very similar to one another and almost indistinguishable from the recurrent parent. Pollen fertilities of the backcross hybrids ranged from 80 to 90 percent.

The seed set in the backcrosses was 70 percent when the triploid was female parent as well as when it was used as male parent. These equivalent fertilities, and the chromosome numbers of reciprocal backcross hybrids, indicate that meiosis is probably similar in megasporogenesis and microsporogenesis.

In Aster as in Hyacinthus, aneuploidy does not result in increased sterility, nor in phenotypic change. ROTHWELL (unpublished data) has found a similar situation in *Claytonia virginica* L. in that aneuploid and polyploid Claytonias are usually highly fertile. Moreover, aneuploids are not morphologically distinct despite extreme variation in chromosome number.

Natural triploid. A natural triploid was collected in 1949 at Yellowwood State Forest in Brown County, Indiana. It is not completely certain whether it is a hybrid between *A. shortii* and *A. cordifolius* or whether it represents a cross between diploid and tetraploid *A. shortii.* That the former situation probably obtains is suggested by the absence of diploid *A. shortii* in southern Indiana as well as the fact that the plant was collected in a mixed population of *A. shortii* and *A. cordifolius.* Configurations at MI were within the range of chromosome associations found in the artificial triploids. Only 21 second anaphase nuclei were counted but the lowest chromosome number found was 14. If the univalents divided once and were distributed at random at the other division, lower gametic chromosome numbers would be expected. The natural triploid is 80 percent fertile, and morphologically similar to *A. shortii.*

DISCUSSION

The unexpectedly high fertility of the triploid hybrids may be due to any one, or some combination, of a number of factors.

1. Viable gametes would contain between 9 to 18 chromosomes if the univalents and trivalents were distributed at random. This situation obtains in *Hyacinthus orientalis* (DARLINGTON 1929), and to a certain extent in Aster.

2. Viable gametes would contain the haploid or diploid number of chromosomes if there were a constant pattern of univalent distribution. This situation

applies to the Canina roses in which the univalents are absent from the functional male gametes but are retained in the functional eggs (FAGERLIND 1940). In triploid Datura, it is believed that nonrandom orientation of trivalents leads to a significant increase in the frequency of n and 2n gametes (SATINA and BLAKESLEE 1937a, b). SEARS (1953) has also noted a tendency for most or all univalents to go together in Triticum-Haynaldia hybrids which, with elimination of gametes with low chromosome numbers, yields an increased frequency in higher chromosome numbers in later generations.

3. The univalents might divide twice at meiosis thus increasing the frequency of higher chromosome numbers, and virtually eliminating gametic chromosome numbers which approximate the haploid condition. The chromosome complement would vary, however, depending upon the number of univalents and trivalents.

There have been observations in various materials which indicate that the univalents may undergo two successive divisions at meiosis. CLAUSEN (1926) reported this situation in certain Viola hybrids although cytological studies did not extend beyond metaphase II. Other *sporadic* occurrences of two successive univalent divisions have been cited by DARLINGTON (1930) in the triploid cherry *Prunus avium nana,* by ERLANSON (1929) in the triploid hybrid *Rosa blanda* × *carolina,* in Raphanobrassica hybrids by KARPECHENKO (1927), in Brassica hybrids by MORINAGA (1929), in Saccharum hybrids by BREMER (1923), and in Ribes hybrids by MEURMAN (1928).

FEDERLEY (1913, 1931) reported the *regular* occurrence of this phenomenon in Pygaera hybrids. Meiosis in these moth hybrids is unique in several respects. There is little chromosome pairing at spermatogenesis and regularity of pairing at oogenesis of F_1 hybrids. Univalents may be eliminated at meiosis in the oocytes of backcross hybrids but not at meiosis of the F_1 hybrid females. Although some univalents may be eliminated at spermatogenesis, they will usually undergo two successive divisions in male hybrids. The hybrid derivatives are partially fertile regardless of variation in chromosome number. Later studies of Dicranura and Drepana hybrids failed to reveal the Pygaera-type meiosis (FEDERLEY 1943, 1949).

Calculations of the frequencies of various gametic chromosome numbers were made on several assumptions, including whether there are one or two univalent divisions, and these were compared with the observed frequency of chromosome numbers in anaphase II nuclei of the three triploid F_1 hybrids (fig. 7). It is quite apparent that the observed data fit with the frequencies calculated on the basis of two successive univalent divisions. The absence of classes of gametes containing fewer than 13 chromosomes immediately suggests a mechanism whereby the chromosome number is increased. However, from table 2 it is seen that there is a significant increase in the frequency of 14-chromosome gametes and a concurrent decrease in the 17-chromosome class. The same tendency is noted for the 15- and 18-chromosome classes. These results may be due to several factors: (1) not all the univalents divide twice; (2) there is a greater error in counting the higher chromosome numbers; (3) some univalents lag and are lost; and (4) more nuclei with fewer

chromosomes are counted since there is less doubt in interpretation. If nonrandom orientation of trivalents were a contributing factor, then one would expect a concurrent increase in both the higher and lower numbers. Since the frequency distributions calculated on the basis of one division of univalents show a peak at 13- and 14-chromosomes, it is conceivable that the observed high frequency of 14-chromosome gamete nuclei is due to occasional univalents which divide but once. However, the other factors cannot be eliminated from consideration.

Although it seems quite certain that univalents undergo two successive divisions at microsporogenesis (and probably megasporogenesis) in Aster triploids, it has not definitely been established that these are both equational divisions. SEARS (1952) found that univalents in hexaploid wheat usually

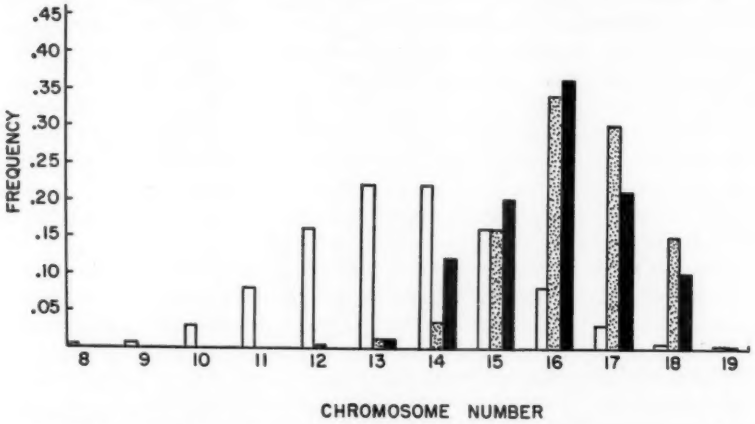


FIGURE 7.—Comparison of the expected frequency of gamete chromosome numbers with (a) only one division of univalents and random distribution (open bar), or (b) two divisions of all univalents (stippled bar), and (c) the observed frequencies (black bar).

misdivide and never separate equationally at both meiotic divisions. The observations of two divisions of the univalents in the species cited above, do not necessarily imply that both divisions are equational.

The presence of a tertiary split in the meiotic metaphase chromosome has been claimed from cytological evidence by NEBEL (1941), HUGHES-SCHRADER (1948), and others. If cytological observations are supplemented with genetic data concerning gamete viability, then substantial evidence of a functional tertiary split is possible. However, fertility data in Aster, as in Pygaera, cannot be used unequivocally since there is no apparent phenotypic effect as a consequence of the presence of extra chromosomes between the triploid and tetraploid levels. All Aster hybrids are fertile regardless of the number of univalents and multivalents present. The same situation in Hyacinthus has been interpreted to mean that little chromosome differentiation has occurred with increase in the number of genomes (DARLINGTON 1929). However, the high

fertility of Aster triploids and backcross hybrids strongly suggests that there is little genic unbalance. If the univalents were to misdivide one might expect greater genic unbalance, as a consequence of the production of telocentrics and isochromosomes, than would result with an increase in the number of whole chromosomes. The high fertility of the hybrids, therefore, suggests equational divisions rather than misdivisions of the univalents present. The fact that lagging chromosomes are rarely seen would also imply infrequency of misdivision.

On the evidence presented, it seems likely that the univalents in the F_1 Aster triploids studied do undergo two successive equational divisions. This is believed to be a cytogenetic demonstration of a functional tertiary split in the meiotic metaphase chromosome.

SUMMARY

Data have been presented which indicate that the univalent chromosomes regularly undergo two successive divisions at meiosis. This phenomenon accounts for the increased frequency of higher chromosome numbers in the resultant gametes of the Aster triploids studied, and consequent increased fertility. The chromosome numbers of nine backcross individuals studied were within the range expected on the basis of two divisions of the univalents in the triploid parent. One natural triploid studied exhibited the same behavior as the artificial triploids.

An attempt was made to correlate these findings with the probability of a functional tertiary split in the meiotic metaphase chromosome. The following observations strongly suggest that the univalents do not misdivide, but rather, undergo successive equational divisions.

1. Lagging univalents were rarely seen. The regularity of disjunction of univalent halves implies a regularity in centromere and chromonema separation, which is not necessarily expected with misdivision.

2. The high fertility of the triploids, which implies genic balance, is most likely the result of the increase in number of whole chromosomes to approximate the diploid complement in the gametes, as a consequence of equational divisions of univalents. Misdivisions result in the production of telocentrics and isochromosomes, which would yield greater genic unbalance and decreased fertility.

Thus, it seems likely that the univalents in the F_1 Aster triploids studied complete two successive equational divisions, which is a cytogenetic demonstration of a functional tertiary split in the meiotic metaphase chromosome.

ACKNOWLEDGMENTS

The author wishes to express her sincere appreciation to DR. RALPH E. CLELAND, DR. CHARLES W. HAGEN, JR., and DR. CHARLES B. HEISER, JR. for their suggestions in the preparation of the manuscript.

This work was completed while the author held the KATHRYN MCHALE Fellowship of the American Association of University Women.

LITERATURE CITED

AVERS, CHARLOTTE J., 1953 Biosystematic studies in *Aster*. I. Crossing relationships in the Heterophylli. Amer. J. Bot. In press.

BELLING, J., 1924 The distribution of chromosomes in pollen-grains of a triploid hyacinth. Amer. Nat. **58:** 440–446.

BREMER, G., 1923 A cytological investigation of some species and species hybrids within the genus *Saccharum*. Genetica **5:** 97–148, 273–326.

CLAUSEN, J., 1926 Genetical and cytological investigations on *Viola tricolor* L. and *V. arvensis* Murr. Hereditas **8:** 1–156.

COLLINS, J. L., L. HOLLINGSHEAD and P. AVERY, 1929 Interspecific hybrids in *Crepis*. III. Constant fertile forms containing chromosomes derived from two species. Genetics **14:** 305–320.

DARLINGTON, C. D., 1929 Meiosis in polyploids. II. Aneuploid Hyacinths. J. Genet. **21:** 17–56.

1930 Studies in *Prunus*. III. J. Genet. **22:** 65–93.

DEMOL, W. E., 1921 De l'existence de variétés hétéroploides de l'*Hyacinthus orientalis* L. dans les cultures hollandaises. Diss. Zürich. Arch. Neèrl. Sci. Exacts et Natur. **4:** 18–143.

ERLANSON, EILEEN W., 1929 Cytological conditions and evidences for hybridity in North American wild roses. Bot. Gaz. **87:** 443–506.

FAGERLIND, F., 1940 Sind die Canina Rosen Agamospermische Bastarde? Svensk. Bot. Tidskr. **34:** 335–354.

FEDERLEY, H., 1913 Das Verhalten der Chromosomen bei der Spermatogenese der Schmetterlinge *Pygaera anachoreta, curtula,* und *pigra* sowie einige ihrer Bastarde. Zeit. ind. Abst. u. Ver. **9:** 1–110.

1931 Chromosomenanalyse der reziproken Bastarde zwischen *Pygaera pigra* und *P. curtula* sowie ihrer Rückkreuzungsbastarde. Zeit. Zellforsch. u. mikr. Anat. **12:** 772–816.

1943 Zytologische Untersuchungen an Mischlingen der Gattung *Dicranura* B. (Lepidoptera). Hereditas **29:** 205–254.

1949 Meiosis and intersexuality in reciprocal *Drepana* hybrids (Lepidoptera). Hereditas **35:** 49–65.

HUGHES-SCHRADER, SALLY, 1948 Cytology of coccids (Coccoidea-Homoptera). Advances in Genetics **2:** 127–203.

KARPECHENKO, G. D., 1927 The production of polyploid gametes in hybrids. Hereditas **9:** 349–368.

MEURMAN, O., 1928 Cytological studies in the genus *Ribes* L. Hereditas **11:** 289–356.

MORINAGA, T., 1929 Interspecific hybridization in *Brassica*. II. The cytology of F_1 hybrids of *B. cernua* and various other species with 10 chromosomes. Jap. J. Bot. **4:** 277–289.

NEBEL, B. R., 1941 Structure of *Tradescantia* and *Trillium* chromosomes with particular emphasis on number of chromonemata. Cold Spring Harbor Symp. Quant. Biol. **9:** 7–12.

SATINA, SOPHIE, and A. F. BLAKESLEE, 1937a Chromosome behavior in triploids of *Datura stramonium*. I. The male gametophyte. Amer. J. Bot. **24:** 518–527.

1937b Chromosome behavior in triploid *Datura*. II. The female gametophyte. Amer. J. Bot. **24:** 621–627.

SEARS, E. R., 1952 Misdivision of univalents in common wheat. Chromosoma **4:** 535–550.

1953 Addition of the genome of *Haynaldia villosa* to *Triticum aestivum*. Amer. J. Bot. **40:** 168–174.

THE COMPOUND X CHROMOSOMES IN DROSOPHILA[1]

EDWARD NOVITSKI
Department of Zoology, University of Missouri, Columbia, Mo.

Received June 20, 1953

CHROMOSOMES formed by the union of homologous elements provide a situation in which somewhat more can be learned about the nature of the meiotic phenomena in Drosophila than may be deduced from a study of normal chromosomes. The classic example is, of course, the attached X chromosome; there are, however, five other types of compound X chromosomes theoretically realizable. All five are now known and have been studied to some extent. It is the purpose of this paper to describe the origin and synaptic properties of the newer compounds and to indicate the kinds of information that have come from a study of them.

TERMINOLOGY

There are four ways in which two X chromosomes may be joined to give a compound with two ends. The centromere of the compound may be either median or terminal and, in each case, the two component chromosomes may be in reversed order with respect to each other or they may be arranged in tandem. A simple self-descriptive terminology is achieved if the compounds with median centromeres are referred to as metacentrics and those with terminal or subterminal centromeres as acrocentrics, and if these terms are preceded by tandem or reversed, depending on the relative order of the two components. The four possibilities are, then, the tandem metacentric, the tandem acrocentric, the reversed metacentric and the reversed acrocentric. It should be noted that the attached X chromosome is the reversed metacentric compound; the latter name is introduced here not to supplant the well established attached X designation, but to indicate the structural relation of the attached X to the other types.

Finally, there are two possible compounds in which the two X chromosomes are joined to form a continuous ring. Here also the two components may be in reversed or tandem order. The first is the reversed ring, the second, the tandem ring.

ORIGIN OF THE COMPOUND X CHROMOSOMES

In principle, the detection of the different compounds is much the same. Females heterozygous for the two X chromosomes to be joined are mated to males carrying a sex-linked dominant, as, for instance, Bar. F_1 female progeny are examined for the presence of Bar; those lacking it must have received both

[1] This investigation was supported by a research grant C-1578 from the National Cancer Institute of the National Institutes of Health, Public Health Service.

X chromosomes from the mother. Such exceptions are then tested to determine whether the two X's are free or whether they have been united.

The left column of figure 1 shows how, by simple crossing over in heterochromatic regions, most of these types might be synthesized. To indicate the relative order of the two chromosomes making up the compound, the letter p represents homologous regions at one end of the components, and the letter d the homologous regions at the opposite end. The nature of the attachments shown in figure 1 is obvious in most cases. The formation of the compound rings (lines 5 and 6) demands the union of the two free ends of a metacentric or acrocentric type. The tandem ring described below has been formed by a crossover between the distalmost heterochromatic region of the tandem acrocentric and a very short arm attached thereto specifically for the purpose of providing opportunity for an exchange to give this product. The reverse ring, on the other hand, might be formed (line 5) from an attached X provided with distal heterochromatin such that if pairing occurred in reverse order, as it might if the heterochromatic regions were derived from different sources, exchange would transform the attached X into a reversed ring. It was contemplated that it might be necessary to employ irradiation to effect this transformation, but before any attempt was made to produce this ring, it appeared spontaneously in a stock of the tandem ring, presumably by crossing over between the basal and interstitial heterochromatin in such a way as to invert one of the component chromosomes, in this particular case, the one in normal order.

The pairing relationships of these types are shown in the right column of figure 1. For simplicity the sister strands are omitted. The three reversed types pair by a simple foldback of the chromosome whereas the tandem types all pair spirally.

The attached X and the tandem metacentric (i.e., tandem attached) X chromosomes have received considerable attention previously and will not be considered in this work.

THE REVERSED ACROCENTRIC COMPOUND X CHROMOSOME

This chromosome type was first described by MULLER (1943, 1944) and later by VALENCIA, MULLER and VALENCIA (1949) and has been referred to by them as the double X chromosome. Its pairing properties are like those of an ordinary attached X chromosome (figure 1), the essential difference being its terminal centromere. The reversed acrocentrics derived by the above workers carried a heterozygous inversion, dl-49, which impairs the usefulness of these chromosomes for a study of crossing over.

Several new chromosomes of this type have been derived in a crossover experiment in which females heterozygous for In(1)*sc*^{8} and a normal chromosome, with *sc cv v f* as markers and with the long arm of the Y chromosome attached as an extra arm, were mated to *y B* males. Two F_1 *B* non-yellow males appeared; they were interpreted as cases in which a crossover between the distal heterochromatin of In(1)*sc*^{8} and the proximal heterochromatin of

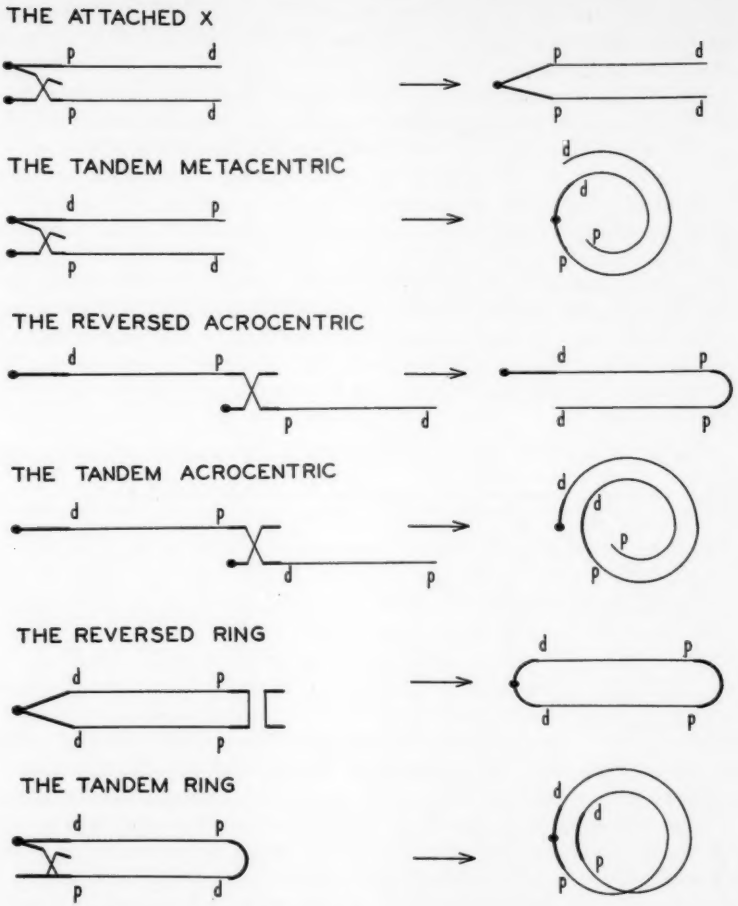


FIGURE 1.—The six structurally different types of compound X chromosomes. The left column shows how each might be derived and the right column illustrates their synaptic properties. The letters p (proximal) and d (distal) simply represent the regions located proximally and distally, respectively, on the normal chromosome to indicate the relative order of the two components. (Other possibilities, genetically different but synaptically similar, are revealed by turning this figure upside down.)

the normal X gave rise to a fragment carrying the small distal portion of the X not included in the sc^8 inversion. An egg carrying such a crossover product, which would have the normal allele of yellow, when fertilized by an X-bearing sperm, would give rise to the *B* males. Because this was a crossover experiment in which only the male progeny were identifiable as crossover types, the female class had been disregarded, but the appearance of the *B* males led to

the examination of the progeny for matroclinous females, a class which might have the crossover product complementary to the fragments, i.e., a reversed acrocentric compound.

Of 8,906 F_1 females examined, 9 were matroclinous, and of these, 4 were fertile and appeared to represent cases of attachment. Two of these were tested extensively, with the results given in table 1 in columns ND1 and ND9.

The consequences of exchange in this compound have previously been pointed out by MULLER and co-workers. Single exchanges will produce no detectable effect if both strands involved are part of the same compound chromatid, but will produce second anaphase bridges when they come from different chromatids. Homozygosis in the progeny for mutants heterozygous in the mother is achieved only by double, or higher, exchange. When crossing over occurs freely, one expects a depressed number of female progeny, as well as a relatively infrequent occurrence of homozygosis. It is rather interesting to note that in both the above cases, mutants originally present in the normal

TABLE 1

Progeny produced by matings of females carrying reversed acrocentric compound X chromosomes to Bar males. The three different series are of different origin; only ND18 is heterozygous for all three recessives.

	ND1	ND9	ND18
+ ♀♀	1479	524	2320
B ♂♂	1974	621	3412
cv v ♀♀	5	0	23
cv ♀♀	4	0	6
v ♀♀	27	0	77
f ♀♀	0	0	65
v f ♀♀	0	0	67
cv v f ♀♀	0	0	15

chromosome appeared to be absent from the compound (*f* in ND1 and *cv v f* in ND9) and since the tests were made immediately after the recovery of the compound, it seems likely that the mutants were lost coincidently with its formation. Such a loss would require a double exchange, in addition to the single heterochromatic exchange furnishing the initial attachment; it should be considered, therefore, that the exchanges giving rise to the reversed acrocentric may be somewhat more complicated than the scheme presented above implies.

Additional information on the spontaneous origin of reversed acrocentrics has been collected by MR. LARRY SANDLER of this department who has kindly consented to the inclusion of his data here. Of 15,575 female progeny from a cross of *sc cv v f car*/sc^8 females × *B* males, 4 matroclinous exceptions were found. These proved to be simply nondisjunctional. However, a parallel cross using an X of normal sequence but with the long arm of the Y chromosome basally (*sc cv v f* · Y^L), gave 9,985 female progeny of which 13 were exceptional. Three of these were sterile or infertile, 5 were nondisjunctional and 5 had reversed acrocentric compound X chromosomes. This suggests that the presence, at the basal region, of material derived from the Y chromosome

facilitates the spontaneous occurrence of the kind of exchange necessary for the formation of this type. Of the five compounds, three were heterozygous for all the mutant alleles, one was homozygous for *v* and *f*, and another for *f* only, the latter two paralleling the homozygosity for the wild type alleles characteristic of the first two analyzed. His results from tests of one of these newly derived reversed acrocentrics (ND18), heterozygous for *cv*, *v* and *f*, are given in column 3 of table 1.

THE TANDEM ACROCENTRIC COMPOUND X CHROMOSOME

The tandem acrocentric compound was constructed in the manner indicated on line 4 of figure 1, i.e., by recovering the compound formed after attachment between the proximal heterochromatin of one chromosome and heterochromatin located distally in a second. The chromosome selected to provide the proximal heterochromatin was In(1)*sc*^{4} and the other, providing the distal heterochromatin was the X-Y chromosome. The X-Y chromosome is made up of an inverted X chromosome, with the short arm of the Y chromosome attached to the free end of the X and the long arm of the Y forming a second arm of the chromosome (LINDSLEY and NOVITSKI 1950).

Accordingly, females of the constitution *y sc*^{4} *car m w*^{a}/In(1)dl-49, *y w lz*^{4} were mated to males carrying the X-Y chromosome. F_1 females heterozygous for the In(1)*sc*^{4} and X-Y chromosomes were irradiated and mated to *B* males and their progeny examined for non-Bar females. Of a total of 14,542 F_1 females counted, 10 were non-Bar, and of these nine were fertile. Further tests showed that six were simply nondisjunctional and three carried newly arisen compound X's. Two of them behaved like attached X's. The third, however, proved to be a tandem acrocentric compound X chromosome.

Reference to figure 2 and table 2 indicates the nature of the argument that this chromosome is, in fact, a tandem acrocentric compound. The large number of patroclinous males comes from the nullo-X eggs, which should amount to half of the total. The + females carry the compound X unaffected by exchange. The rest of the types are crossovers. It is interesting to note that a common single crossover product of the tandem acrocentric is a single chromosome. The region of the exchange responsible for the transformation of the tandem acrocentric into a single chromosome may be identified when the chromosome is recovered in an F_1 male, but cannot be identified when recovered in the female progeny since they carry the paternal X chromosome, which is marked only with Bar. The 110 heterozygous Bar females recovered in set 1 therefore represent single chromosomes recovered in the female sex compared to 119 recovered in the male. The total number of tandem acrocentrics recovered is only 16. Other types, occurring singly, and interpreted as a consequence of more complicated exchanges, are omitted from the table.

It was realized that the deficiency of the tandem acrocentric class in the progeny might result, in part at least, from a viability effect of an excessive amount of heterochromatin, since this compound would carry, adjacent to the centromere, the long arm of the Y chromosome and the basal heterochromatin

of the proximal component, an interstitial heterochromatic segment at the junction of the two components, and finally any heterochromatin present at the distal end of the In(1)sc^4 chromosome. In addition, females with this chromosome would ordinarily have the Y chromosome of their father. Females carrying this chromosome were therefore mated to X-Y males in successive generations in order to remove the Y chromosome contributed by the father.

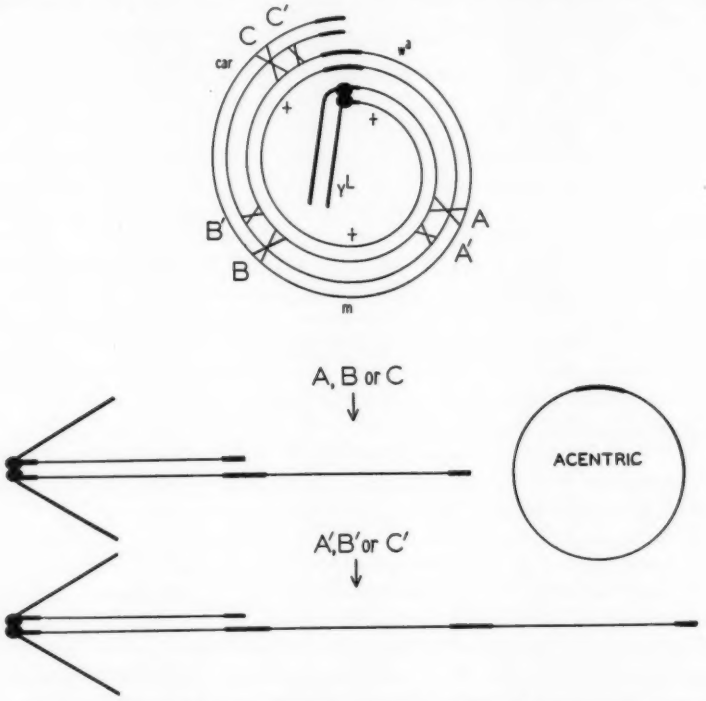


FIGURE 2.—Exchange in the tandem acrocentric compound heterozygous for *car*, *m* and w^a, showing the specific regions involved in the production of the various types of single chromosomes, the lower diagram illustrates the nature of the difference between the two distinct types of single exchanges.

The results from such females lacking a Y chromosome are given under set 2 in table 2.

The effectiveness of removing the Y chromosome is shown by the increase in the percentage of tandem acrocentric-bearing progeny recovered in the second set, 10.6%, as opposed to 4.1% in the first set. Confirmation of this is provided by a comparison of the numbers of male and female progeny carrying single chromosomes generated by crossing over. In set 1, they are recovered equally frequently (119 males : 110 females) but in set 2, where the females

are heterozygous for the X-Y chromosome, and consequently have the equivalent of an extra Y chromosome, the ratio of males to females is 185:88. On the basis of these results all subsequent experiments involving compound X chromosomes have involved the use of the X-Y chromosome.

The inequality of the numbers of single and compound chromosomes recovered merits comment. The higher frequency of single chromosomes might appear to be a manifestation of the type of nonrandom disjunction which has been found in certain cases where heteromorphic dyads are produced by crossing over (NOVITSKI 1951). However, it can easily be shown that, although the configurations after crossing over are of the heteromorphic dyad type, the data do not indicate any nonrandomness in this case. The argument may be most simply stated in the following way. For each type of exchange producing a single chromatid and leaving the compound intact, there should be another

TABLE 2

The types of progeny produced by females with tandem acrocentric chromosomes heterozygous for the mutants w^a, m and car, mated to sc^{SI} B InS w^a sc^a (Muller-5) males (set 1) and X-Y, v f B males (set 2).

		Set 1	Set 2
1. Patroclinous males	Muller-5	212	....
	X-Y, *v f B*	...	367
2. a. Noncrossovers	+ ♀	16	77
b. Single crossovers in			
region A	*m car* ♂	58	100
region B	*car* ♂	53	81
region C	+ ♂	8	4
Total singles recovered in ♂♂		119	185
Singles in A, B or C recovered in ♀♀	*B*/+ ♀	110	88
c. Double crossovers in regions B and C	*m* ♀	3	5

type occurring equally frequently (in the absence of chromatid interference) which produces a single and a triple chromatid (see figure 2). It should be noted that although only single exchanges are considered here, the net result from double exchanges is equivalent to that from singles. Since the triple chromosomes cannot be recovered, a correction must be made to account for this class by doubling the number of tandem acrocentrics recovered (or by halving the number of singles). On this basis, the best comparison of the frequencies of the two types comes from set 2 and is given by the ratio of the males carrying a single chromosome to double the number of females with a tandem acrocentric, i.e., 185 to 164.

A more precise method of testing for the presence of nonrandomness involves the derivation of a simple equation from the algebraic expressions for the consequences of each type of exchange, which then expresses nonrandomness, *c*, directly as a function of the frequencies of the observed classes. This had

been done previously for the tandem metacentric compound (NOVITSKI 1951); the formula for the tandem acrocentric differs only in that two of the coefficients are changed:

$$c = \text{Single chromosomes} / (4 \times \text{Patroclinous } \delta\delta + 2 \times \text{tandem acrocentrics} - \text{Single chromosomes}) + \text{C.F.}$$

where C.F. represents a correction factor dependent upon the relative frequencies of the tetrads of ranks 1 and 2, and upon c itself, but amounting to a few percent, at most.

When the best estimate available for determining these classes is substituted, a c value of 0.48 is obtained, which, after the addition of the small correction factor, brings c so close to 0.5 that there can be little doubt that nonrandomness does not appear to be operating here.

THE TANDEM COMPOUND RING

The tandem compound ring was formed in the manner indicated on line 6 of figure 1. In order to facilitate crossing over between the distalmost segment of the tandem acrocentric compound and the long arm of the Y chromosome which forms the short arm of this chromosome, the fragment from $T(1,4)B^s$ carrying the centromere of the X and the segment up to and including the Bar locus was added. A crossover attaching this basal piece of the translocation removes the normal allele of yellow found at the tip of the Y^L in the X-Y chromosome used in making up the tandem acrocentric. Many offspring of irradiated females carrying the tandem acrocentric and the B^s fragment were examined, and a small number were found which were simultaneously yellow and Bar; these proved to carry the desired attachments. Such a tandem acrocentric compound X chromosome now has an extensive homologous region at the base and at the tip, arranged in such an order as to produce a tandem ring, if crossing over should occur between them, an event which would be easily detectable since such a ring chromosome would lack the Bar locus. One such yellow non-Bar female appeared in the progeny of $y\,B$ mothers; the presence of a large double sized ring was confirmed cytologically. All experiments have been performed with the progeny of this female.

The pairing configuration of this ring is illustrated in figure 3. Crossing over of nonsister chromatids can give rise to a number of different products, these are given in table 3. The designations of types of exchange in this table follow that shown in the figure.

The tandem ring chromosome as finally derived is homozygous for yellow and miniature. There are no heterozygous mutants present suitably located for making observations on frequencies of homozygosis, or relative frequencies of exchange in different regions of the chromosomes. The mutant allele w^a is heterozygous, but its position is so unfavorable from the standpoint of providing information that it has been disregarded. Instead, the number of single chromosomes produced is used as a measure of the frequency of exchange and a simple estimate of the total number of eggs with X chromosomes, or prod-

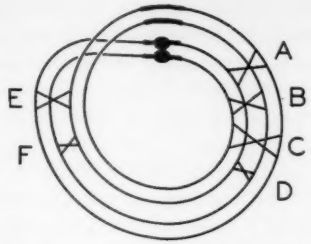


FIGURE 3.—The essentially different types of exchange theoretically expected in the tandem compound ring configuration. A catalog of the consequences after exchange is found in table 3.

ucts thereof, in any experiment is made by doubling the number of patroclinous males, just as in experiments involving other compound X chromosomes.

The first tests of females carrying the tandem ring, *y m*, × X-Y *v f B* males produced the following types in the F_1: 8224 *v f B* males; 1844 *v f B/y m* females; 2095 *y m* males and 735 *y m* females. There are several points about these data worth mentioning: (1) The discrepancy between the numbers of single rings recovered in females and males, 1844 to 2095; (2) the gross inequality between the number of single and double rings recovered, 3939 to 735, and (3) the much greater number of patroclinous males recovered than ring-bearing individuals.

The greater frequency of ring-bearing males than females may be interpreted as a depression of the female class because of the excess heterochromatin present on the X-Y chromosome as well as that present in duplicate adjacent to the centromere on the single ring, an effect analogous to that discussed in the section on the tandem acrocentric. For this reason, the appropriate figure for

TABLE 3

Expected consequences of exchange of ranks 0, 1 and 2 in the tandem ring chromosome. Certain types which represent duplicates of those given below are omitted when such omission does not lead to an error in calculating the frequencies of expectation of the distinctively different types.

Rank	Crossovers involved	Relative frequency	Products after second anaphase division
0	None	E_0	Double ring + Double ring
1	A, B or E	$\frac{1}{2}E_1$	Single ring + acentric ring + Double ring
	C, D or F	$\frac{1}{2}E_1$	Bridge
2	A + E	$1/8E_2$	Double ring + Double ring
	D + F	$1/8E_2$	Double ring + Double ring
	A + F	$1/8E_2$	Single ring + Triple ring
	C + E	$1/8E_2$	Single ring + Triple ring
	B + E	$1/8E_2$	Single ring + acentric rings + Single ring
	B + F	$1/8E_2$	Bridge + acentric ring
	C + F	$1/8E_2$	Bridge + acentric ring
	D + E	$1/8E_2$	Bridge + acentric ring

the recovery of single rings will be arrived at by doubling the number of males carrying rings rather than by addition of the male and female classes.

From table 3, it is apparent that single and double exchanges are similar in that (assuming no chromatid interference) 25% of the products are single rings, 25% are double rings and 50% give rise to lethal zygotes. These lethal zygotes result from the formation of dicentrics or triple rings; the latter would be recoverable only in superfemales, a class which has not been seen. Since single rings are recovered in both sexes in the F_1 irrespective of the type of sperm fertilizing the egg, and the compound rings only after fertilization of the egg by a nullo-X sperm, the total number of compound rings must be multiplied by a factor of two. The comparison is then 4190 single rings to 1470 compound rings. It should be noted that if the tandem ring were about 37% as viable as the single ring produced by it, the number of ring-bearing eggs accounted for (single rings + tandem rings + dicentrics = 4190 + 4190 + 2(4190) = 16,760) would be in good agreement with double the number of patroclinous males ($2 \times 8224 = 16{,}448$), which would suggest that no special assumptions need be made to account for the behavior of the tandem ring chromosome. It will be shown below, however, that there is reason to believe that the viability of the tandem ring may not be this low.

While most of the patroclinous males undoubtedly arise in the usual way, i.e., from the fertilization by X-bearing sperm of that half of the egg nuclei not receiving an X, it seemed conceivable that if certain types of second anaphase bridges persisted as bridges, a nullo-X egg might be formed by the exclusion of the bridge, or any breakage product of it, from the egg nucleus. This might happen for either of two reasons: (1) the bridges formed by crossing over in the tandem ring are unlike ordinary anaphase II bridges, in that two chromatids connect each centromere and (2) a new type of bridge could appear as a result of the intertwining of the ring chromatids. The distinction between the usual and the two new possible origins of nullo-X eggs is easily made by the simple addition of a genetically marked fragment which ordinarily disjoins from the compound X, for the nullo-X eggs formed in the usual way will carry the fragment whereas the latter two types will not.

The fragment used is that described by NOVITSKI (1951) and designated as FR2. It is made up of the long arm of the Y chromosome and the distal uninverted segment from In(1)sc^8 carrying the normal allele of yellow. Accordingly. females of the constitution *y m*, tandem ring/FR2, y^+ were mated to *y B* males. The progeny consisted of 2530 *B* males, 254 *y B* males, 766 *y B*/*y m* females, 913 *y m* males, 245 *y m* females and 3 *m* females. The last class is nondisjunctional, the egg nucleus having included within it both the ring and the fragment; the small size of this class testifies to the regular separation of these two chromosomes. Consequently we may regard the 2530 *B* males as coming from the usual nullo-X class and the 254 *y B* males as a new nullo-X type, probably produced in one or the other of the two ways postulated above.

The results can now be analyzed in much the same way as in the preceding

case. If we multiply the best figure for the occurrence of single rings by four, we arrive at a value of 3652, an unusually high value since four times the number of single rings recovered in the males should not exceed the number of patroclinous males (2530). It is clear that the discrepancy between the single rings and the double rings persists in this experiment.

THE REVERSED COMPOUND RING X CHROMOSOME

During the course of an experiment conducted by MR. STANLEY ZIMMERING of this department involving the tandem ring, a number of females being tested produced, not the usual array of types including single chromosomes, but only matroclinous daughters and patroclinous sons, that is, they behaved as though they carried ordinary attached X chromosomes. It was clear that the tandem ring chromosome had, in some way, stabilized itself, a condition that would have been selected for rather strongly in the stock cultures from which the females were taken.

Cytological examination revealed the nature of this stabilization. Prophases of larval neuroblasts showed that a double ring was still present and, in the salivary gland chromosomes, the two components could be seen to be arranged in reverse order, with the yellow ends of both components now located adjacent to the centromere. Cytologically, then, this chromosome satisfies the definition of the reversed compound ring and the genetic results agree with this determination.

While the reversed ring chromosome is of interest in its own right, one arising from the tandem ring is of particular interest since it throws some light on the question of the viability of the tandem ring, for the essential difference between the two lies, not in their gene content, but in the order of the components. Whether the two are identical genetically cannot, of course, be determined, since this would depend on the nature of the event leading to the transformation. It seems most likely that this change took place by, first, pairing of the interstitial heterochromatic segment located between the two components and the heterochromatin on one side or the other of the centromere and, second, the occurrence of an exchange in such a direction as to invert that component found in normal order (see fig. 4).

The consequences of exchange in the reversed ring chromosome parallel those in the reversed acrocentric X chromosome. No new recoverable chromosome types (like single chromosomes) are produced regularly, as they are in the tandem ring. In the absence of heterozygous mutants, exchanges can be considered to consist of two types: either they have no detectable effect, or they produce bridges, and, barring chromatid interference, these two types should occur equally frequently in tetrads of all ranks except, of course, zero. One should, therefore, observe a reduction in the number of reversed rings recovered, because of exchange and also because of any inviability inherent in the ring itself.

From a mating of females of the constitution double ring, *y m*/FR2 y^+ to X-Y, *y B* males, the F_1 consisted of 3951 *B* males, 391 *y B* males, 1443 *y m*

females and 13 *m* females. The last class is nondisjunctional. If the total number of X eggs is taken as twice the number of fragment bearing males, ($2 \times 3951 = 7902$), then 2912 ($= 2 \times 1456$) of these are accounted for as double ring-bearing eggs, the doubling of the class being necessary since only that half of the ring-bearing eggs fertilized by nullo-X sperm are recoverable. But, except for the no exchange tetrads, there should be as many inviable zygotes from bridge formation as there are recoverable reversed rings. If all tetrads were to have at least one exchange, then the corrected figure for the number of reversed rings would be 2×2912 or 5824. The figure for the viability of females carrying the reversed ring then becomes 5824/7902, or .74 of the *B* male class. On the other hand, if crossing over were completely suppressed in this configuration, the relative viability would then be 2912/7902 or .37. The frequency of exchange for this particular compound is not known, but unless it is basically different in its behavior from the other compound chromosomes,

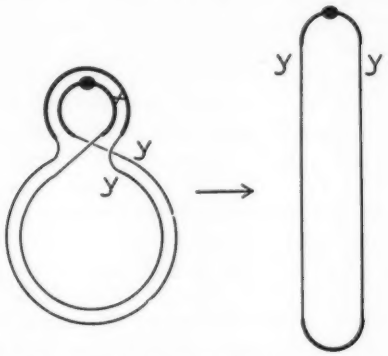


FIGURE 4.—The heterochromatic pairing relationships and crossover event that could convert the tandem ring into the reversed ring.

and particularly from the tandem ring from which it originated, it may be surmised that the viability value for the reversed ring is closer to the higher limit than the lower.

DISCUSSION

For each of the new compound X chromosomes described here, there is presented a set of presumably typical data which indicates a gross parallel between the theoretically expected behavior and the observed. In no case have the analyses been sufficiently detailed to determine whether there is complete agreement between the expectations based on the usual assumptions of crossover analyses and the observations; this is particularly true of the two compound rings since the observations were made without benefit of heterozygous loci, and it might be pointed out that a study of crossing over using chromosomes devoid of markers must leave some questions unanswered. For the tandem ring, the production of single rings is used as the measure of exchange.

But for the reversed ring, the only criterion of exchange is the depression of the female class in the progeny and this depression, unfortunately, is equally interpretable as a simple viability effect.

There are, nevertheless, several points that may be made on the basis of the data presented above. First, it is of interest to note that the tandem acrocentric compound described here does not behave in the anticipated way with respect to nonrandom disjunction. It had been shown previously (NOVITSKI 1951) that the analysis of crossover data from experiments involving heteromorphic homologues leads to the conclusion that when a heteromorphic dyad is produced by crossing over, the smaller of the two chromatids is included in the functional egg nucleus of Drosophila more often than the larger. Since the tandem acrocentric also produces heteromorphic dyads after exchange, one might expect it to behave similarly. The results using this chromosome indicate that it does not. There appears to be no satisfactory explanation for this behavior of the tandem acrocentric at the present time. One possibility is that the presence of the long arm of the Y chromosome as an additional arm on both chromatids (see fig. 2) minimizes the relative difference between the two dissimilar chromatids.

The regular recovery of single ring chromosomes from the tandem ring with a frequency not far from expectation is of particular interest. Simultaneously with the production of single centric rings, acentric rings also may be formed and the evidence indicates that they must have little, if any, influence on the recoverability of the single centric rings. If crossing over were the consequence of a breakage of two nonsister chromatids, followed by an uncoiling and refusion of broken chromatids, the result would be an interlocked complex with the rings intertwined around each other. In the absence of any special faculty of the chromosomes to free themselves permanently from such interlocks (unlike the breakage of chromatids at the succeeding anaphase which would lead to interlocks in subsequent anaphases), the acentric fragments, as passive participants in such interlocks, might lead to an appreciable loss of the rings generated by crossing over. Since the data deny any appreciable loss of single centric rings, it seems reasonable to suggest that the mechanism of crossing over may be other than that described above.

In this connection, it should be pointed out that the genetic consequences of interlocking of ring chromatids after crossing over and the implications of the absence of a deficiency of rings with respect to theories of crossing over have been considered by others but that in previous work involving rings, all rings were centric, leaving open the possibility that interlocks formed in this way would simply break at anaphase, followed by a refusion of the broken chromatids to reconstitute normal rings. It was for the elucidation of this point that the tandem ring was synthesized.

SUMMARY

Two X chromosomes of *D. melanogaster* may be joined together in six structurally distinct ways. Four of the combinations have two free ends. When

the centromere is located medially, as it is in the attached X, the compound is referred to as a metacentric, and when the centromere is located terminally (or subterminally), it is called an acrocentric. In each case, the order of the two X chromosome components may be tandem or reversed (mirror image). Furthermore there are two compound rings, one with the two components in tandem and the other with the two components reversed with respect to each other. The six types of compounds are, then, the attached X (reversed metacentric), the tandem metacentric, the reversed acrocentric, the tandem acrocentric, the tandem ring and the reversed ring. The reversed types synapse by a simple foldback of the two components, whereas the tandem types synapse spirally. All the tandem compounds are unstable and produce single chromosomes by crossing over.

All six types are now known; data are presented indicating that the new types, the acrocentrics and the rings, behave in a way not inconsistent with the simple predictions based on the present knowledge of crossing over in Drosophila. The tandem ring produces single centric and single acentric chromosomes by crossing over. The absence of an appreciable deficiency of the single centric rings suggests that the centrics and acentrics are not ordinarily interlocked after crossing over, a condition which would have some bearing on theories of the mechanism of crossing over.

ACKNOWLEDGMENTS

The writer wishes to extend his thanks to his colleagues at the University of Missouri for their helpful criticisms during the course of the work and in the preparation of the manuscript and to PROF. A. H. STURTEVANT for his suggestions on the problem of nomenclature.

LITERATURE CITED

LINDSLEY, D. L., and E. NOVITSKI, 1950 The synthesis of an attached X Y chromosome. Drosophila Inf. Ser. **24**: 84–85.

MULLER, H. J., 1943 A stable double X chromosome. Drosophila Inf. Ser. **17**: 61–62.
1944 High primary non-disjunction of the insertional double-X. Drosophila Inf. Ser. **18**: 57.

NOVITSKI, E., 1951 Non-random disjunction in Drosophila. Genetics **36**: 267–280.
1952 The genetic consequences of anaphase bridge formation in Drosophila. Genetics **37**: 270–287.

VALENCIA, R. M., H. J. MULLER and J. I. VALENCIA, 1949 Formation of attached X's by reverse crossing over in the heterochromatic regions. Drosophila Inf. Ser. **23**: 99–102.

GENETICS SOCIETY OF AMERICA

EXECUTIVE COMMITTEE

President, J. T. PATTERSON — *Vice-President*, R. A. BRINK

Past Presidents, J. W. GOWEN, ROY E. CLAUSEN

Secretary, C. P. OLIVER
University of Texas
Austin 12, Texas

Treasurer, N. H. GILES, JR.
Yale University
New Haven, Connecticut

"The Genetics Society of America is organized to provide facilities for association and conference among students of heredity and for the encouragement of close relationship between workers in genetics and those in related subjects. All persons actively interested in any field of genetics shall be eligible to active membership. Candidates for membership must be recommended by two members of the Society. Members shall be elected by the Executive Committee."—Constitution, Article I.

Any one interested in joining the Society should write to the Secretary.

The Genetics Society publishes annually the Records of the Society containing the program and abstracts of the Annual Meeting and others items of interest to members, including a membership list at periodic intervals. Total membership of the Society was 950 on September 10, 1952. Copies of the Records may be obtained from the Treasurer for $1.00. The Genetics Society has a representative on the Editorial Board of GENETICS, Tracy M. Sonneborn.

SUSTAINING MEMBERS

The Genetics Society of America established in 1949 a new type of membership, Sustaining Members. The constitution provides that "All organizations interested in any field of genetics shall be eligible to sustaining membership." The dues for sustaining members are $50.00 a year. Money contributed by sustaining members is not used for running expenses of the Society, but is used for special purposes as decided by the Executive Committee. The Executive Committee at a meeting in Ithaca, New York on December 29, 1953, voted unanimously that $1,000 from the sustaining membership account be allocated to GENETICS toward its publication costs.

The following organizations became sustaining members of the Society in the years indicated:

Associated Seed Growers
New Haven, Conn.—1950

Bear Hybrids Corn Co.
Decatur, Ill.—1950

W. Atlee Burpee Co.
Philadelphia 32, Pa.—1950

DeKalb Hybrid Seed Co.
DeKalb, Ill.—1949

Ferry-Morse Seed Co.
Detroit 31, Mich.—1949

Funk Brothers Seed Co.
Bloomington, Ill.—1950

Kimber Farms
Niles, California—1953

Moews Seed Co.
Granville, Ill.—1949

Nichols Poultry Farm
Kingston, N. H.—1950

Pioneer Hi-Bred Corn Co.
Johnston, Iowa—1949

This page is donated by GENETICS to the GENETICS SOCIETY OF AMERICA. The society and the journal are separate organizations with a common bond of interest in furthering the science of heredity.

INFORMATION FOR CONTRIBUTORS

Contributions to GENETICS may be in the field of genetics proper, or in any scientific field, if of such a character as to be of primary genetic interest.

The length of manuscripts will be limited to twenty printed pages, including tables and illustrations. Longer papers may be published by special vote of the Editorial Board and if the author agrees to bear the cost of the additional pages. In case unusually extensive tabular material cannot be printed, it will be kept on file on request, provided two copies are furnished by the author.

The *Galton and Mendel Memorial Fund* established in 1923 from donations of biologists and other persons interested in the progress of biological discovery is available to be applied toward the cost of reproducing illustrations and of printing expensive tables and formulae.

Authors should submit two copies of their manuscripts to the Editors. Manuscripts should conform to the general usage in GENETICS, particularly in regard to references to literature, arrangement of "Literature Cited" and inclusion of a summary. In case of "Acknowledgments," these should follow the "Summary." Footnotes should be avoided in the text whenever possible and their content be inserted, in parentheses, after the appropriate sentence. Footnotes to tables should be marked with an asterisk, dagger, or other symbol so as not to be confused with the figures in tables. Legends for figures and plates should be type-written separately from the illustrations. In addition to the original illustrations photographic copies should accompany the manuscript. Tables also should be type-written separately. The metric system should be used whenever possible.

Galley proofs and page proofs will be sent to authors, except in case of authors resident overseas who, in general, will receive galley proofs only. Authors should leave forwarding directions whenever they are to be away from the address sent with the manuscript.

Reprints may be ordered at the time galley proof is returned. The author will be billed for them in accordance with the schedule of charges appearing on the order blank. Copies of the schedule may be obtained from the Editorial Office.

Manuscripts and editorial correspondence should be addressed to the Editors of GENETICS, Genetics Building, University of Wisconsin, Madison 6, Wisconsin.

INFORMATION FOR CONTRIBUTORS

Contributions to GENETICS may be in the field of genetics proper, or in any scientific field, if of such a character as to be of primary genetic interest.

The length of manuscripts will be limited to twenty printed pages, including tables and illustrations. Longer papers may be published by special vote of the Editorial Board and if the author agrees to bear the cost of the additional pages. In case unusually extensive tabular material cannot be printed, it will be kept on file on request, provided two copies are furnished by the author.

The *Galton and Mendel Memorial Fund* established in 1923 from donations of biologists and other persons interested in the progress of biological discovery is available to be applied toward the cost of reproducing illustrations and of printing expensive tables and formulae.

Authors should submit two copies of their manuscripts to the Editors. Manuscripts should conform to the general usage in GENETICS, particularly in regard to references to literature, arrangement of "Literature Cited" and inclusion of a summary. In case of "Acknowledgments," these should follow the "Summary." Footnotes should be avoided in the text whenever possible and their content be inserted, in parentheses, after the appropriate sentence. Footnotes to tables should be marked with an asterisk, dagger, or other symbol so as not to be confused with the figures in tables. Legends for figures and plates should be type-written separately from the illustrations. In addition to the original illustrations photographic copies should accompany the manuscript. Tables also should be type-written separately. The metric system should be used whenever possible.

Galley proofs and page proofs will be sent to authors, except in case of authors resident overseas who, in general, will receive galley proofs only. Authors should leave forwarding directions whenever they are to be away from the address sent with the manuscript.

Reprints may be ordered at the time galley proof is returned. The author will be billed for them in accordance with the schedule of charges appearing on the order blank. Copies of the schedule may be obtained from the Editorial Office.

Manuscripts and editorial correspondence should be addressed to the Editors of GENETICS, Genetics Building, University of Wisconsin, Madison 6, Wisconsin.

GENETICS Vol. 39 No. 1 JANUARY, 1954

CONTENTS OF PRECEDING ISSUES

MAY, 1953

HOUSE, VERL L., The interaction of mutants affecting venation in *Drosophila melanogaster*. I. Interaction of Hairless, engrailed and cubitus interruptus.

LANDAUER, WALTER, Genetic and environmental factors in the teratogenic effects of boric acid on chicken embryos.

JOHNSON, B. LENNART, Evidence for irregularity in crossing over of the S locus in the eversporting type of *Matthiola incana* (L.) R. Br.

STEINITZ-SEARS, LOTTI M., and E. R. SEARS, Chiasmata and crossing over in a dicentric chromosome in wheat.

SCHWARTZ, DREW, Evidence for sister-strand crossing over in maize.

DUNN, L. C., and S. GLUECKSOHN-WAELSCH, Genetic analysis of seven newly discovered mutant alleles at locus T in the house mouse.

WALLACE, BRUCE, J. C. KING, CAROL V. MADDEN, BOBBIE KAUFMANN and E. C. MCGUNNIGLE, An analysis of variability arising through recombination.

JULY, 1953

HOUSE, VERL L., The interaction of mutants affecting venation in *Drosophila melanogaster*. II. Additive and pattern effects of combinations of Hairless, engrailed and cubitus interruptus.

BAKER, WILLIAM K., V-type position effects of a gene in *Drosophila virilis* normally located in heterochromatin.

LEFEVRE, G., JR., F. J. RATTY, JR. and G. D. HANKS, Frequency of Notch mutations induced in normal, duplicated and inverted X-chromosomes of *Drosophila melanogaster*.

HOROWITZ, N. H., and MARGUERITE FLING, Genetic determination of tyrosinase thermostability in Neurospora.

KIRBY-SMITH, J. S., and D. S. DANIELS, The relative effects of X-rays, gamma rays and beta rays on chromosomal breakage in Tradescantia.

STINSON, HARRY T., Cytogenetics and phylogeny of *Oenothera argillicola* Mackenz.

DE SERRES, FREDERICK J., and NORMAN H. GILES, The effect of radiation dose fractionation on chromosome aberration frequencies in Tradescantia microspores. I. Studies with X-rays.

GILES, NORMAN H., FREDERICK J. DE SERRES and ALVIN V. BEATTY, The effect of radiation dose fractionation on chromosome aberration frequencies in Tradescantia microspores. II. Studies with fast neutrons.

SEPTEMBER, 1953

KYLE, W. H., and A. B. CHAPMAN, Experimental check of the effectiveness of selection for a quantitative character.

BREWBAKER, JAMES L., Oppositional allelism in diploid and autotetraploid *Trifolium hybridum* L.

WALLACE, BRUCE, and CAROL MADDEN, The frequencies of sub- and supervitals in experimental populations of *Drosophila melanogaster*.

DOBZHANSKY, THEODOSIUS, and BORIS SPASSKY, Genetics of natural populations. XXI. Concealed variability in two sympatric species of Drosophila.

LAUGHNAN, JOHN R., The effect of the *sh*$_2$ factor on carbohydrate reserves in the mature endosperm of maize.

LEVINTHAL, C., and N. VISCONTI, Growth and recombination in bacterial viruses.

DUNN, L. C., and S. GLUECKSOHN-WAELSCH, The failure of a t-allele (t^8) to suppress crossing over in the mouse.

PROUT, TIMOTHY, CHARLES HUEBSCHMAN, HOWARD LEVENE and FRANCIS J. RYAN, The proportions of nuclear types in Neurospora heterocaryons as determined by plating conidia.

NOVEMBER, 1953

GOWEN, JOHN W., JANICE STADLER, H. H. PLOUGH and HELEN N. MILLER, Virulence and immunizing capacity of *Salmonella typhimurium* as related to mutations in metabolic requirements.

SIEGEL, R. W., A genetic analysis of the mate-killer trait in *Paramecium aurelia*, variety 8.

LEVINE, MYRON, The diverse mate-killers of *Paramecium aurelia*, variety 8: their interrelations and genetic basis.

MCCLINTOCK, BARBARA, Induction of instability at selected loci in maize.

STEBBINS, G. L., and FUNG TING PUN, Artificial and natural hybrids in the Gramineae, tribe Hordeae. VI. Chromosome pairing in *Secale cereale* × *Agropyron intermedium* and the problem of genome homologies in the Triticinae.

EATON, ORSON N., Heterosis in the performance of mice.

KAPLAN, WILLIAM D., The influence of Minutes upon somatic crossing over in *Drosophila melanogaster*.

Abstracts of papers presented at the 1953 Meetings of the Genetics Society of America.

SINGLE NUMBERS $1.50 ANNUAL SUBSCRIPTION $8.00

obtainable from

GENETICS, INC., THE SCIENCE PRESS, BOX 749, LANCASTER, PA.